REMARKS

This amendment is being filed in response to the Office Action mailed November 18, 2002. Applicants thank the Examiner for rejoining claims 8 to 13 with elected claims 1 to 7 for examination in the application. By the present amendment, claim 13 has been canceled herein without prejudice. Applicants maintain the right to prosecute canceled claim 13 in any related application claiming the benefit of priority of the subject application. New claims 28 to 39, directed to the subject matter deleted from amended claims 1 to 13, have been added. Accordingly, upon entry of the amendment, claims 1 to 12 and 28 to 39 are under consideration.

Regarding the Amendments

The amendments to the specification were made to address several informalities or are supported by the specification. The title has been amended and the specification has been amended to include a description for Figure 12, as requested by the Examiner. The amendment to the title addresses an informality. The amendment to insert the description for Figure 12 is supported, for example, at page 35, lines 22-24, which discloses what Figure 12 shows.

The amendments to claims 1, 2 and 5 to 12 were made to address informalities or are supported by the specification. The amendment to delete the recitation of "galectin-3 binding polypeptide, or galectin-3 receptor binding polypeptide" was made in view of new claims 28 to 39. The amendment to claim 5 was made in response to the Examiner's suggestion. The amendment to claim 11 to recite "decreasing" is supported, for example, at page 17, lines 17-24. The amendment to claim 12 to recite "increasing" was made to conform the preamble of the claim with the body of the claim, and is also supported, for example, at page 16, lines 16-18, and at page 17, lines 15-17.

Thus, the amendments to the specification and claims address informalities or are supported by the specification and, therefore, do not add new matter. Accordingly, entry thereof is respectfully requested.

Regarding the New Claims

New claims 28 to 39 are directed to the language deleted from claims 1 to 12, as presently amended. Claims 28 to 39 are therefore supported by originally filed claims 1 to 12.

Thus, as new claims 28 to 39 are supported by the specification, no new matter has been added and entry thereof is respectfully requested.

I. REJECTIONS UNDER 35 U.S.C. §112

The rejection of claims 1 to 13 under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description is respectfully traversed. The Examiner states that allegedly "the specification and claims do not indicate what distinguishing attributes are shared by members of the genus" or "any guidance as to what changes should be made." Thus, allegedly "one skilled in the art cannot reasonably visualize or predict critical amino acid residues which would structurally characterize the genus of galectin-3 proteins claimed."

Claims 1 to 13 are adequately described. Nevertheless, in order to expedite prosecution of the application, claim 13 has been canceled herein without prejudice. The rejection will therefore be addressed as it may pertain to claims 1 to 12 and 28 to 39.

First, various sequences of the recited galectin-3 molecules were known in the art at the time of the invention. As evidence of this knowledge, submitted herewith are Exhibits A-C (Barondes *et al.*, J. Biol. Chem. 269:20807 (1994); Robertson *et al.*, Biochem. 29:8093 (1990); and Cherayil *et al.*, Proc. Natl. Acad. Sci. USA 87:7324 (1990), respectively). Exhibit A is a minireview that describes in detail the structure and function of galectins 1-4. Single peptide chains for each of galectins 1-4 are schematically illustrated in Figure 1. Second, the conserved regions of galectins were known in the art. For example, all galectins contain one or two homologous carbohydrate binding domains of about 130 amino acids (Figure 1 and 2). Third, the features that distinguish galectins 1-4 from each other were known in the art. For example, the structure of galectin-3 is distinct from galectins 1, 2 and 4, as illustrated in Figure 1 and 2. Galectin-3 contains a proline-glycine-tyrosine rich repeating domain in addition to a carbohydrate binding domain. Moreover, it was also known in the art that each mammalian galectin has 80-90% homology in the carbohydrate domain (e.g., comparing galectin 3 from mouse, rat and human); however, different galectins (e.g., comparing galectin 1 to 2, 3 to 4 etc.) share only 20-40% identity in the carbohydrate binding domain (see page 20807, second column, second paragraph). Based on the fact that the structural and functional regions of the galectins were known in the art and, furthermore, that the features that distinguish galectin-3 from other

galectins were known, those skilled in the art would be apprised of a galectin-3 genus. Significantly, the authors share this conclusion stating that "it is fairly easy to identify the equivalent lectin in these different species." (see page 20807, second column, second paragraph).

As to galectin-3 in particular, Exhibit B, which describes a human galectin-3 sequence (Figure 1), indicates that galectin-3 molecules are highly conserved between human, rat and mouse, 84% and 82% respectively (see abstract). In the carboxyl-terminal domain, there is 95% identity between the human and murine sequences over a stretch of 70 amino acids (see abstract). Exhibit C, which describes the cloning of human mac-2 (galectin-3; Figure 2), indicates that the cloned hmac-2 cDNA is "highly conserved, with 85% of its amino acid residues similar to those in the murine homolog." Thus, Exhibits B and C further corroborate the high sequence conservation shared among galectin-3 molecules, and therefore, that those skilled in the art would be apprised of a galectin-3 genus.

In sum, in view of the fact that the structurally conserved and functional regions of galectin-3 molecules were known in the art at the time of the invention, and that the structure and function that distinguish galectin-3 molecules from other galectin molecules were also known in the art at the time of the invention, as corroborated by Exhibits A-C, those skilled in the art would be apprised of the genus of galectin-3 molecules. As such, an adequate written description for galectin-3 in claims 1 to 12 and 28 to 39 is provided and the rejection under 35 U.S.C. §112, first paragraph must properly be withdrawn.

The rejection of claims 1 to 13 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is respectfully traversed. The Examiner indicates that claim 5 is allegedly unclear, that the metes and bounds of "galectin-3" allegedly are not known, and that "it is not understood how galectin-3 binding polypeptides, or galectin-3 receptor binding polypeptides....can inhibit migration."

Claims 1 to 13 are clear and definite as written. Nevertheless, solely in order to further prosecution of the subject application and without acquiescing to the propriety of the rejection, claim 13 has been canceled herein without prejudice. In addition, claim 5 has been amended as suggested by the Examiner.

As to the metes and bounds of "galectin-3" as discussed above, those skilled in the art would be apprised of the genus of galectin-3 molecules. As such, the metes and bounds of "galectin-3" are known.

As to how galectin-3 binding polypeptides, or galectin-3 receptor binding polypeptides can inhibit migration, claims 3 and 4 are directed to "stimulating" and "inhibiting" migration. In this regard, the specification discloses that galectin-3 and galectin-3 fragments can stimulate migration (see, e.g., Figures 1 and 5 and 8-11). As to inhibiting migration, a modified inactive galectin-3 that binds to a galectin-3 receptor but does not stimulate migration can inhibit binding of endogenous galectin-3 thereby inhibiting migration. Thus, inactive galectin-3 fragments or that retain galectin-3 receptor binding can inhibit migration. Thus, in view of the foregoing, galectin-3 molecules can either stimulate or inhibit migration.

The specification also exemplifies an antibody that inhibits migration (see, e.g., Figure 2). As to antibodies that stimulate migration, an antibody that binds galectin-3 may stimulate migration by forming galectin-3 oligomers, a process thought to contribute to the ability of galectin-3 to stimulate migration (see, e.g., page 10, lines 15-25). Thus, by promoting galectin-3 olgiomerization migration is stimulated. Likewise, as disclosed in the specification, an agonist antibody that binds galectin-3 receptor and functions like galectin-3 thereby stimulates migration in a manner similar to galectin-3 (see, e.g., page 16, lines 11-12). Alternatively, as disclosed in the specification an antibody or agent that binds galectin-3 receptor may stimulate galectin-3 receptor crosslinking, thereby stimulating migration (see, e.g., page 13, lines 18-22 and page 20, lines 1-3). Thus, in view of the foregoing, galectin-3 binding polypeptides and galectin-3 receptor binding polypeptides can either stimulate or inhibit migration.

In view of the amendment to claim 5 and the foregoing remarks, claims 1 to 12 and 28 to 39 are clear and definite. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph be withdrawn.

II. REJECTIONS UNDER 35 U.S.C. §102 and 103(a)

The rejection of claims 1 to 13 under 35 U.S.C. §102(b) as allegedly anticipated by Hughes *et al.* (Glycobiology 4:5 (1994)) is respectfully traversed. The Examiner indicates that

Hughes *et al.* allegedly describe that "extracellular matrix remodeling would be necessary for extravasation and hence the lectin ligation of surface glycoproteins in early recruitment of inflammatory macrophages, such as those present at inflammatory sites...may be significant."

Claims 1 to 13 are not anticipated by Hughes *et al.* Nevertheless, in order to expedite prosecution of the application, claim 13 has been canceled herein without prejudice. The rejection will therefore be addressed as it may pertain to claims 1 to 12 and 28 to 39.

In order for a rejection to be proper under 35 U.S.C. §102 a single prior art reference must disclose each element of the claim under consideration. *In re Spada*, 15 USPQ 2d 1655 (Fed. Cir. 1990), *In re Bond*, 15 USPQ 2d 1566 (Fed. Cir. 1990). "The identical invention must be shown in as complete detail as is contained in the....claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236 (Fed. Cir. 1990).

In the present case, Hughes *et al.*, *inter alia*, fail to disclose the invention in as complete detail as contained in the claims. For example, Hughes *et al.* state that "macrophages taken from sites of non-immunologically mediated inflammation....*are believed* to be newly recruited cells that have recently entered the extravascular space." Hughes *et al.* then discusses the alterations of expression of numerous proteins following ligation of galactosyl residues, stating that "[a]mong the proteins, the secretion of which is increased, are....TNF-α and a 35.6 kDa collagenase, *suggesting that* ligation of macrophage surface glycoproteins by a-galactose-binding lectins *may* induce secretion of enzymes involved in the turnover of extracellular matrices." Finally, Hughes *et al.* state that "Extracellular matrix remodeling would be necessary for extravasation and hence the lectin ligation of surface glycoproteins in early recruitment of inflammatory macrophages, such as those present at inflammatory sites of thioglycollate-primer animals, *may* be significant." [Emphasis added] Thus, no less than two qualified statements precede the statement relied upon by the Examiner to assert that Hughes *et al.* teach the claimed invention. In fact, the very statement relied upon as allegedly describing the claimed invention includes the term "may." Given that qualifying words are used throughout this paragraph, e.g., "believed, suggesting, may," and the like, Hughes *et al.* is at best speculating.

Furthermore, each of these statements made by Hughes *et al.* is necessary for the subsequent statements to be made. If a preceding statement is incorrect, then the statement that follows can not be true. Here, two qualified statements precede the sentence by Hughes *et al.* that the PTO has relied upon in making the rejection. Thus, if one or both of these statements is

untrue, then the statement relied upon by the PTO cannot be true. Accordingly, it cannot objectively be said that two qualified statements which must be accurate in order for a third qualified statement to be true describes the invention in as complete detail as contained in the claims. In this regard, one skilled in the art, in view of Hughes *et al.*, would not have known that galectin-3 could be used to modulate migration of a cell as in claims 1 to 12, let alone that galectin-3 binding polypeptide, or galectin-3 receptor binding polypeptide could be used to modulate migration of a cell as in claims 28 to 39.

The Examiner appears to agree with Applicant's position. For example, in the Office Action it is acknowledged that "Hughes *et al.* does not teach the use of galectin-3 to modulate the migration of macrophages to tumor sites or infection." [Last paragraph bridging Pp. 4-5] It is further acknowledged that "Hughes *et al.* do not specifically state that galectin-3 is involved in cell migration, nor do they specifically teach methods for modulating immune cells at tumor or infection sites, such as in vivo treatment. Hughes *et al.* also do not teach the use of anti-galectin antibodies." [page 5, last paragraph] Yet, in spite of these admissions, the Patent Office inexplicably reasons that "one of ordinary skill in the art would immediately envision that galectin-3 could be used to recruit (i.e. migrate) cells." [Last paragraph bridging Pp. 4-5]

Thus, in view of the fact that Hughes *et al.* at most make a series of speculative statements including the statement relied upon by the Patent Office as allegedly teaching the claimed methods, it cannot objectively be said that Hughes *et al.* describe the invention in as complete detail as contained in the claims, as is required for a rejection to be proper under 35 U.S.C. §102. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §102(b) be withdrawn.

The rejection of claims 1 to 13 under 35 U.S.C. §103(a) as allegedly unpatentable over Hughes *et al.* in view of Hawkins *et al.* (U.S. Patent No. 5,869,289) is respectfully traversed. The Examiner acknowledges that "Hughes *et al.* do not specifically state that galectin-3 is involved in cell migration, nor do they specifically teach methods for modulating immune cells at tumor or infection sites, such as in vivo treatment. Hughes *et al.* also do not teach the use of antigalectin antibodies." [page 5, last paragraph]

In order for a rejection to be proper under 35 U.S.C. §103, *inter alia*, there must have been a motivation to modify or combine the references at the time of the invention; the

combination of references must teach or suggest each and every element of the claimed invention; and there must have been a reasonable expectation of success at the time of the invention. Here, *inter alia*, there would not have been a reasonable expectation of success at the time of the invention in view of the cited art.

In particular, for example, Hughes *et al.* is merely speculative and, therefore, cannot fairly be said to provide a reasonable expectation of success. Furthermore, Hughes *et al.* do not teach or suggest that any galectin molecule could modulate cell migration. Moreover, as to claims 28 to 39, Hughes *et al.* fail to teach or suggest using a galectin-3 binding polypeptide, or galectin-3 receptor binding polypeptide in order to modulate migration of a cell that expresses a galectin-3 receptor. Thus, in view of these deficiencies, one skilled in the art would not have had a reasonable expectation of success, as required for a rejection to be proper under 35 U.S.C. §103, in view of Hughes *et al.*

Hawkins *et al.* also do not provide a reasonable expectation of success. First, Hawkins *et al.* is a patent directed to human galectin-8, which is not being claimed (see abstract). Furthermore, Hawkins *et al.* do not demonstrate that galectin-8 or any other galectin for that matter, let alone galectin-3, can modulate cell migration. In fact, galectin-3 is only mentioned in the background section and Hawkins *et al.* fail to teach or suggest that galectin-3 can be used to modulate cell migration. At most, Hawkins *et al.* indicate that "galectin-3 inhibits cell adhesion by binding to laminin....plays a role in inflammation...concentrates in the nucleus of certain cell types during proliferation....and is elevated in tumors, suggesting galectin-3 plays a role in metastasis." (paragraph 3, lines 41-50) Moreover, as to claims 28 to 39, Hawkins *et al.* fail to teach or suggest using a galectin-3 binding polypeptide, or galectin-3 receptor binding polypeptide in order to modulate migration of a cell that expresses a galectin-3 receptor. Thus, in view of the foregoing it cannot objectively be said that Hawkins *et al.* teach or suggest using galectin-3, galectin-3 binding polypeptide, or galectin-3 receptor binding polypeptide in order to modulate cell migration, let alone provide the skilled artisan with a reasonable expectation of success. As such, Hawkins *et al.* do not provide that which is missing from Hughes *et al.*

Thus, in view of the above deficiencies of Hughes *et al.* and Hawkins *et al.*, one skilled in the art would not have a reasonable expectation of success of producing claims 1 to 12 and 28 to 39. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §103(a) be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 1 to 12 and 28 to 39 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4093.

Please charge any additional fees, or make any credits, to Deposit Account No. 03-3975.

Respectfully submitted,

Date: May 19, 2003

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Minireview

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Galectins

STRUCTURE AND FUNCTION OF A LARGE FAMILY OF ANIMAL LECTINS*

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Lectins are proteins that bind to specific carbohydrate structures and can thus recognize particular glycoconjugates among the vast array expressed in animal tissues. Most animal lectins can be classified into four distinct families (1): C-type lectins (including the selectins); P-type lectins; pentraxins; and galectins (2), formerly known as S-type or S-Lac lectins (1). The purpose of this short review is to provide a framework for integrating the rapid increase in knowledge of the diversity, structure, and function of the galectins. While the emphasis here is on mammalian galectins, important advances are also being made in studies of galectins in other species, including nematode (3) and sponge (4).

Structural Classification and Properties of Galectins

Members of the galectin family are defined by two properties: shared characteristic amino acid sequences and affinity for β-galactoside sugars (2). Individual mammalian galectins are named by consecutive numbering. Presently four mammalian galectins, galectin-1, -2, -3, and -4, have been well characterized. Of these, galectin-1 and galectin-3 have been rediscovered in several species and in different contexts, leading to multiple names and the potential for confusion. Fortunately, a consensus on naming has recently been reached (2) so that galectin-1 will be used for the same lectin that has been described in human, bovine, rat, and mouse tissues as L-14-I, L-14, galaptin, and BHL among other names; and galectin-3 will be used for the same lectin that has been described in human, dog, rat, and mouse tissues as Mac-2, εBP, CBP-35, CBP-30, and L-29 among other names. The general designation of the genes encoding galectins is *LGALS* (lectin, galactoside-binding, soluble), and gene numbering is being kept consistent with the numbering of the proteins, so that *LGALS1* encodes galectin-1, etc. (2). In humans, *LGALS1* and *-2* have been mapped to the q12-q13 region of chromosome 22 (5), and *LGALS3* has been mapped to chromosome 1p13 (6).

The overall structures of galectin-1, -2, -3, and -4 are shown schematically in Fig. 1. Galectin-1 and -2 are homodimers composed of subunits of approximately 130 amino acids (Fig. 2). Each subunit folds as one compact globular domain (7, 8) as shown in Fig. 3. Galectin-3 and -4 include one or two such domains, as well as others (Fig. 1). The shared domain has been referred to as the carbohydrate-binding domain.

The sequence of each carbohydrate-binding domain has been shown to be mainly encoded by 3 exons (9–11) as shown in Fig. 2. Most of the residues that are conserved among galectins (marked by *orange dots* below sequences in Fig. 2) are found in the sequence encoded by the middle one of these three exons (*middle part* of Fig. 2). This sequence includes four contiguous β-strands and intervening loops in the structure of galectin-1 (8) and galectin-2 (7) (colored *blue* in Fig. 3 and marked by *blue bars* in Fig. 2) and contains all

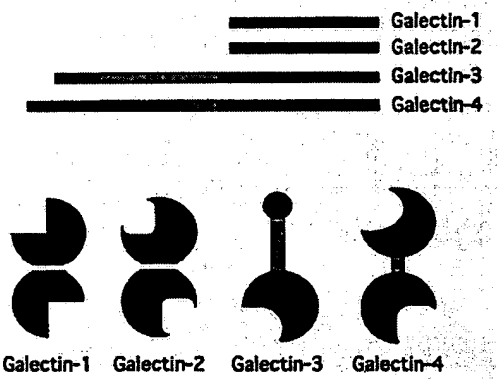


FIG. 1. **Schematic of the overall structures of galectin-1, -2, -3, and -4.** The proteins are shown schematically as linear diagrams corresponding to single peptide chains (*top*) and as assembled proteins (*bottom*). The carbohydrate-binding domains of about 130 amino acid residues are *blue*, the proline-, glycine-, and tyrosine-rich repeating domain of galectin-3 (about 100 residues) and link peptide of galectin-4 (about 30 residues) are *orange*, and the N-terminal domain of galectin-3 (about 30 residues) is *green*.

residues that interact directly with a carbohydrate ligand (marked by *light blue dots* above the sequences in Fig. 2 and shown in detail in Fig. 4). The importance of some of these residues for carbohydrate binding activity is also supported by site-directed mutagenesis (12, 13). Deletion of sequences encoded by the other two exons that encode the carbohydrate-binding domain also impairs activity (12).

The amino acid identity in the carbohydrate-binding domains among different known galectins from one mammalian species ranges from about 20 to 40% (14). The identity of the same galectin from different mammalian species is 80–90%, so that it is fairly easy to identify the equivalent lectin in these different species. In contrast, it is more difficult to relate galectins from non-mammalian species to those from mammals. For example, two chicken galectins, C-16 and C-14, have been cloned and sequenced (3). Both have subunits of about 15 kDa and are 48% identical in sequence. Since each is about 50% identical to galectin-1 and 38% identical to galectin-2, it is not clear on the basis of sequence how they correspond with the mammalian galectins.

In addition to the carbohydrate-binding domain, galectin-3 has a short N-terminal domain and an intervening proline, glycine, and tyrosine-rich domain (Fig. 1). The latter domain consists of repeats of 7–10 amino acids with a consensus sequence of $PGAYPG(X)_{1-4}$ (where X is any amino acid). The number of repeats varies with species, and hence the size of galectin-3 varies from 26.2 kDa in man to 30.3 kDa in dog (15). Neither of the additional domains is required for carbohydrate binding activity, which is preserved by the C-terminal domain generated upon proteolysis of the intact protein (16, 17). Galectin-3 is isolated as a monomer but undergoes multimerization on binding to surfaces that contain glycoconjugate ligands, and the N-terminal half of the protein is required for this property (16, 17). Galectin-4, a monomer of about 36 kDa, contains two carbohydrate-binding domains within a single peptide chain. These domains are connected by a link region that is homologous to the repeating domain of galectin-3 (14).

In addition to the four well characterized mammalian galectins already mentioned, three related mammalian proteins have been identified and tentatively called galectin-5, -6, and -7. Publications describing them are in preparation, and partial sequences are already deposited in GenBank for galectin-5 (accession number L21711) and galectin-7 (accession numbers L07769 and U06643).

A number of interesting proteins have structural similarity to galectins but do not fulfill the definition of galectins. The Charcot-Leyden protein, a major constituent of eosinophils, has been found to share significant sequence homology with galectin carbohydrate-binding domains (18) but lacks some of the critical residues that

* This minireview will be reprinted in the Minireview Compendium, which will be available in December, 1994. The molecular graphics images were produced using the MidasPlus, RibbonJr, Neon, and Ilabel programs from the Computer Graphics Laboratory, University of California, San Francisco (supported by National Institutes of Health Grant RR-01081).

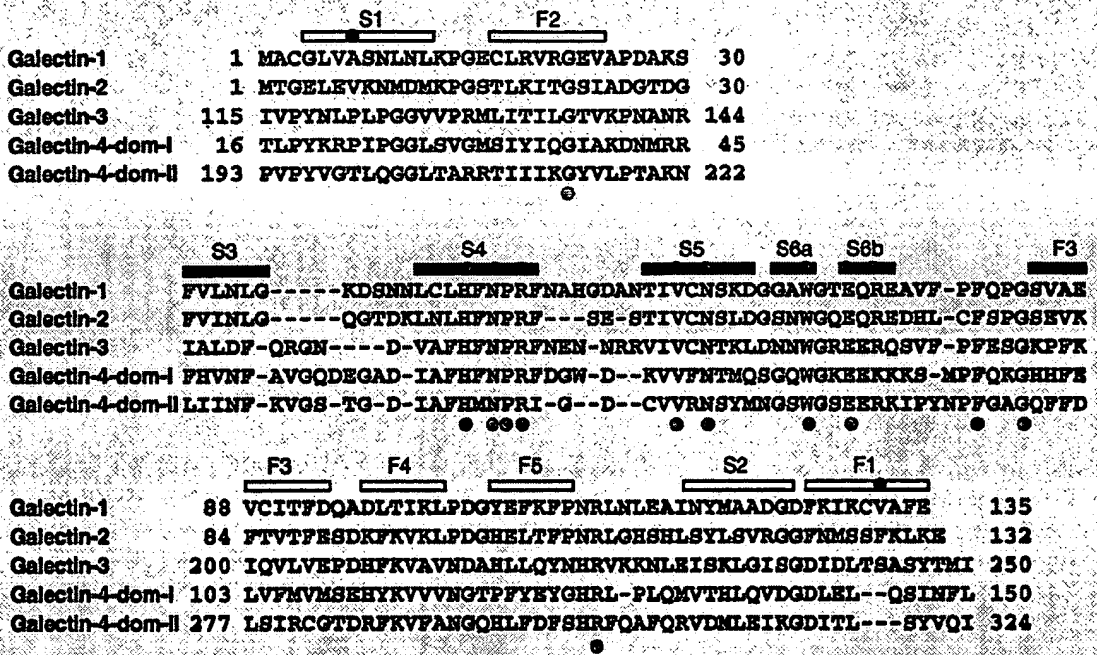
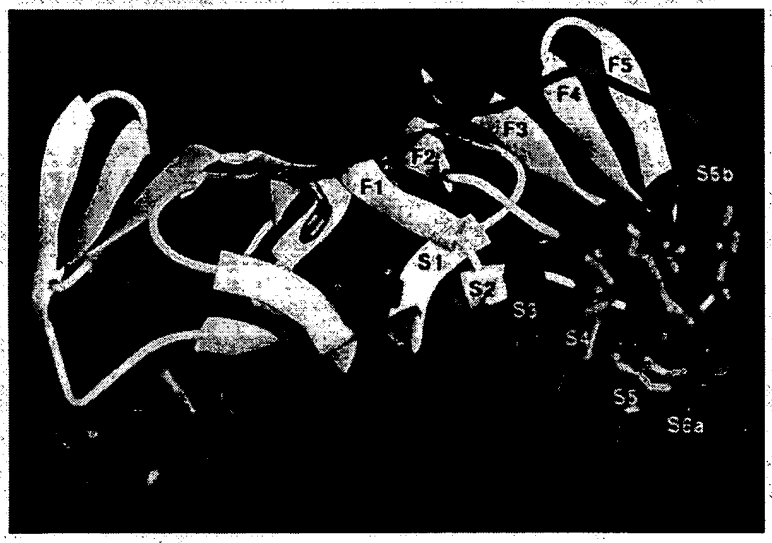


FIG. 2. **Sequence of the carbohydrate-binding domains of galectin-1, -2, -3, and -4.** The sequences are presented in three parts reflecting the genomic organization of galectin-1, -2, and -3, with the *middle part* corresponding to the exon encoding the carbohydrate-binding site (exon III for galectin-1 and -2 (9), and exon V for galectin-3 (10, 11)). The *symbols* above the sequences refer to structural features of galectin-2 (7) and are *colored* as in Fig. 3. The *bars* indicate sequences in the different β-strands (S1–5, S6a, S6b, and F1–5) in galectin-2 (as labeled in the right subunit in Fig. 3) and are colored *blue* (if encoded by exon III) or *white*. The residues indicated with *dots* above the sequences directly interact with the carbohydrate (*light blue*) or contribute to the dimer interface (*purple*). Residues that are identical in all five of the carbohydrate-binding domains shown (and most other galectins (14)) are indicated by *orange dots* below the sequences. The sequences are for human galectin-1 (9), galectin-2 (41), galectin-3 (79), and rat galectin-4 carbohydrate-binding domains I and II (14).



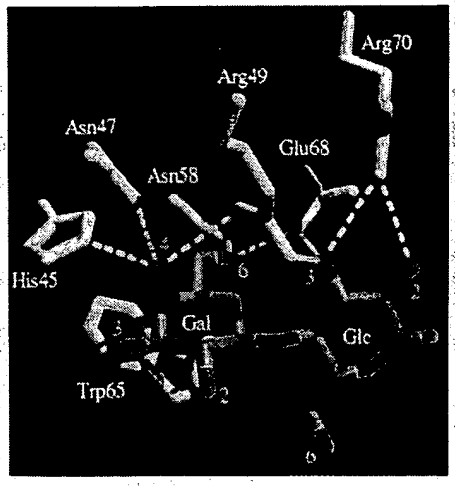


FIG. 3 (*left*). **X-ray crystal structure of human galectin-2 (7).** The figure shows a dimer of two globular carbohydrate-binding domains (*blue* and *white ribbon diagram*) with bound lactose (*green stick representation* with *red* ring oxygens). The part of each domain (subunit) encoded by exon III (*middle part* of Fig. 2) is shown in *blue* and the other parts in *white*. The dimer interface is in the middle and is highlighted by *purple coloring* of the major contributing residues (Val and Ile). Each subunit consists of a sandwich of two β-sheets of 6 and 5 strands. In the subunit to the right the strands of each sheet are labeled S1–5, S6a, and S6b (strand 6 is interrupted by a β-bulge), and F1–5. The amino acid side chains interacting with the carbohydrate are displayed as *light blue stick figures*. In the right subunit the carbohydrate-binding site is facing toward the viewer, whereas in the left subunit the carbohydrate-binding site is facing away from the viewer.

FIG. 4 (*right*). **Fine structure of interaction between galectin-2 and lactose (7).** The sugar residues are shown in *green* with *red* oxygen atoms and position numbers. The amino acid side chains interacting with the saccharide are shown in *light blue*. The principal hydrogen bonds between the amino acid side chains and the sugar residues are shown as *yellow dotted lines*. Figs. 3 and 4 were generated using UCSF MidasPlus (80).

may be required for sugar binding and has no known carbohydrate binding activity. Subunits of the legume lectins (19) and serum amyloid P-component (a pentraxin with lectin activity) (20) have the same topology and very similar tertiary structure as the galectin carbohydrate-binding domains but show no significant sequence homology. This suggests that galectins are a subset of a larger group of proteins sharing conserved folding motifs. Members of this group may include other pentraxins, as well as recently discovered membrane proteins in the endoplasmic reticulum-Golgi that show sequence homology to the legume lectins (21).

Carbohydrate Binding Specificity of Galectins

All mammalian galectins whose carbohydrate binding specificity has been examined in detail recognize the same structural determinant on lactose and related β-galactosides (14, 22–26). X-ray crystallography confirms this interaction in the case of galectin-1

(8) and galectin-2 (7) (Fig. 4). The major interaction is with the galactose residue in lactose. However, interaction with the glucose residue in lactose is also significant, as reflected in the 100-fold higher affinity for lactose compared with galactose (22).

Further substitutions on the β-galactose residue differentially affect interaction with specific galectins, presumably reflecting differences in β-strands S2–4 (Figs. 2 and 3) which may contribute to an extended binding site (22, 26, 27). Such an extended site might explain the much higher affinity of galectin-3 for blood group A saccharides that are substituted with GalNAcα 1–3 (22). It might also accommodate consecutive *N*-acetyllactosamine residues as found in polylactosaminoglycans, which are particularly good ligands for many galectins (22, 27).

Major Known Sites of Expression of Mammalian Galectins

Galectins are expressed with distinct but overlapping distributions in mammalian tissues. Although there has been a history of immunohistochemical localization of galectins, the recent discovery of potentially cross-reactive galectins complicates the interpretation of these results. Nevertheless, based on studies that have identified specific galectins by protein or nucleic acid sequence, it is clear that galectin-1 is abundant in skeletal, smooth, and cardiac muscle (28), motor and sensory neurons (29, 30), thymus, kidney, and placenta (28). In contrast, expression of galectin-3 is highest in activated macrophages, basophils, and mast cells (31, 32), some epithelial cells (*e.g.* intestine and kidney) (33–35), and in some sensory neurons (30, 36). In many tissues galectin synthesis is activated only during particular developmental or physiological stages (3, 28, 37–39), and low levels have been found for additional cell types, such as fibroblasts (40). Much less is known about the distribution of galectin-2, which is expressed in hepatomas (41), or galectin-4, which is expressed in intestinal epithelium (14).

Non-classical Secretion of Galectins

Although some galectins clearly are secreted, no galectins (mammalian or other) show any evidence of a typical secretion signal peptide (3, 4, 42, 43). This would normally imply that galectins are confined to the cytoplasm. However, galectin-1 and -3 are abundant not only in the cytosol but also extracellularly (37, 39, 44); and there is direct evidence that these galectins are externalized by non-classical secretory mechanisms (31, 33, 44, 45). Non-classical secretion of cytosolic proteins that play extracellular roles has also been demonstrated for interleukin-1β, basic fibroblast growth factor, and other proteins (46, 47).

Non-classical secretion of galectin-1 has been best studied in skeletal muscle, where the protein moves from a diffusely intracellular to an extracellular location during *in vivo* development (38, 44, 49). In cultured myoblasts, galectin-1 remains in the cytosol until it is externalized during differentiation, apparently by membrane evagination (44, 48). The lectin can then interact with oligosaccharides on laminin (50) and perhaps other extracellular glycoproteins (51).

Secretion of galectin-3 by macrophages was first inferred when this protein was identified as a major macrophage cell surface antigen, Mac-2 (39). In kidney and polarized intestinal epithelial cells, there is direct evidence for its secretion, again by a non-classical pathway, in this case specifically to the apical surface (33, 45). Secretion increases strikingly in response to stress, such as inflammation (31) or heat shock (33).

There is also evidence for secretion of other galectins. For example, a 14-kDa chicken galectin has been found in intestinal epithelial cells and directly shown to be secreted into the intestinal lumen (52). A galectin in *Xenopus* skin (42) has been shown to be secreted by a specialized holocrine mechanism (53). In this case the cytosolic lectin and some other cytosolic constituents are released by rupture of glandular cells in the skin.

The reason galectins are secreted by non-classical pathways is not known. One possibility is to segregate them from complementary glycoconjugate ligands (externalized by the classical pathway) so that galectins and their ligands interact only after externalization. Another possibility is that, in contrast with the single classical secretion pathway, there may be multiple non-classical secretory mechanisms so that different galectins in a cell might be selectively secreted in response to specific signals, as suggested for other proteins (54). Such specific secretion is known in bacteria where different cytosolic proteins are exported by specific transporters (55).

Ligands for Galectins

Despite the large number of β-galactoside-containing glycoconjugates present in the cellular milieu, few glycoproteins from cell extracts bind to particular galectins *in vitro* (50, 56–61), suggesting that these may be the interactions that are physiologically significant. It is not unreasonable to expect that there will be more than one significant ligand for each galectin, as for other molecules such as neurotransmitters that have many different receptors.

Among naturally occurring glycoconjugates, glycoproteins that contain polylactosamines are especially good ligands for galectins (22, 27). Of these, laminin, a glycoprotein with many polylactosamine chains, has been implicated as a natural ligand for galectin-1 (50, 62, 63) and is also bound by galectin-3 (25, 64). Galectin-1 has also been shown to bind to other glycoconjugates including polylactosamine-rich lysosome-associated membrane proteins (LAMPs) (56, 60) that are sometimes found on the cell surface, a lactosamine-containing glycolipid on olfactory neurons (65), and integrin $\alpha_7\beta_1$ on skeletal muscle cells (51). Galectin-3 is also known to interact with immunoglobulin E (32) and its receptor (66) and to copurify with a 90-kDa secreted glycoprotein (57, 58) found in epithelial cells. Other putative ligands from macrophages have been detected by chemical cross-linking (31).

Biological Functions of Galectins

Given their evolutionary conservation, wide tissue distribution, marked developmental regulation, and abundance in particular tissues, the galectins have been presumed to function in important biological processes. The fact that galectin-1, -2, and -4 are divalent and galectin-3 can form multivalent aggregates suggests that they act by cross-linking carbohydrate chains on cell surfaces and/or in the extracellular matrix. However, direct evidence for particular functions has only recently begun to accumulate even for the best studied galectins, galectin-1 and -3.

The general idea that animal lectins might function in modulating cell-cell and cell-matrix interactions has a long history (37) and has been been well demonstrated for some members of a different lectin family, the selectin subgroup (1, 67) of C-type lectins. Recent studies suggest that galectins, too, participate in modulating cell adhesion but in novel ways that promise to open an entirely new area in cell biology. In addition, there is mounting evidence that galectins also participate in a number of other biological processes and that the function of a given galectin can vary from site to site depending on the nature of available ligands.

Galectin-1 has been shown to either promote or inhibit cell adhesion. In skeletal muscle, where galectin-1 is secreted during differentiation and binds to laminin (50), it inhibits cell-matrix interaction. This is presumably the result of binding of galectin-1 to polylactosamine chains on laminin, which interferes with laminin recognition by the major laminin receptor on myoblasts, integrin $\alpha_7\beta_1$ (51). Consequent inhibition of cell-matrix adhesion has been proposed to have a role in muscle development (50, 51). In contrast, galectin-1 can promote cell-matrix adhesion for other cell types, apparently by cross-linking cell surface and substrate glycoconjugates (27, 60, 65). In addition, there is evidence that galectin-1 might participate in regulating cell proliferation (68–70) and some immune functions (71, 72). However, mice lacking galectin-1 have been engineered, and thus far, no phenotype has been detected (73). Absence of a phenotype is frequently seen with gene knockout experiments and has been attributed to alternative proteins and biological mechanisms that compensate for the missing protein.

Galectin-3 might also play a role in multiple biological processes through interaction with specific ligands. As with galectin-1, binding of galectin-3 to polylactosamine chains on laminin can inhibit cell adhesion (25). On the other hand, affinity of galectin-3 for both IgE and an IgE receptor can trigger activation of mast cells and basophils (66) and play a role in inflammation (32). Upon secretion from intestinal epithelial cells galectin-3 has been postulated to play a role in bacterial colonization through its ability to bind both to mucins and bacteria in the intestinal lumen (33, 74). Galectin-3 might also perform intracellular functions, because it has been identified as a component of ribonucleoprotein particles (75) and is

concentrated in the nucleus during proliferation of some cell types (34, 75). Elevated expression of galectin-3 in tumors has been proposed to play a role in metastasis (76). Overexpression of recombinant galectin-3 in a weakly metastatic fibrosarcoma line resulted in a large increase in metastatic potential (77).

While galectin-1 is not believed to undergo any post-translational modification other than N-terminal methionine cleavage and acetylation, several post-translational modifications of galectin-3 might influence its function. Ser-6 is phosphorylated by casein kinase I (78), and the proline-, glycine-, and tyrosine-rich domain can be degraded by tissue collagenases (15), which would prevent galectin-3 self-aggregation (16, 17).

Future Directions

In the past few years, there has been progress in identifying new galectins in mammals and other species, cloning them, and ascertaining the structural features that determine carbohydrate binding. This work has provided new reagents and opportunities to answer the following questions. How large is the galectin gene family? What are the relevant glycoconjugate ligands for each of these lectins, and what are the physiological functions of galectin binding? What is the significance of the unusual manner of galectin secretion and how is it accomplished? Answering these questions should greatly advance our limited understanding not only of galectins but also of a larger issue, the significance of the complex glycoconjugates that surround all cells.

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Human IgE-Binding Protein: A Soluble Lectin Exhibiting a Highly Conserved Interspecies Sequence and Differential Recognition of IgE Glycoforms†,‡

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ABSTRACT: IgE-binding protein (εBP) refers to a protein originally identified in rat basophilic leukemia cells by virtue of its affinity for IgE. It is now known to be a β-galactoside-binding lectin equivalent to carbohydrate-binding protein 35 (CBP 35). More recently, its identity to Mac-2, a macrophage cell-surface protein, has been established. cDNA coding for human εBP has been cloned from a human HeLa cell cDNA library and contains an open reading frame of 750 base pairs encoding a 250 amino acid protein. Like the rat and murine counterparts, the human εBP amino acid sequence can be divided into two domains with the amino-terminal domain consisting of a highly conserved repetitive sequence (YPGXXXPGA) and the carboxyl-terminal domain containing sequences shared by other S-type lectins. The human εBP sequence exhibits extensive homology to murine and rat εBP with 84% and 82% identity, respectively. The homology is particularly striking in the carboxyl-terminal domain where 95% identity is found between human and murine sequences in a stretch of over 70 amino acids. A survey of εBP mRNA expression from several lymphocyte cell lines revealed that the level of εBP transcription may reflect a relationship between cell differentiation and εBP expression. Finally, human εBP was purified from several human cell lines and shown to possess lactose-binding characteristics and cross-species reactivity to murine IgE. Surprisingly, three different human myeloma IgE proteins did not show reactivity to human εBP. However, after neuraminidase treatment of each human IgE, pronounced binding to εBP was observed, thereby indicating that only specific IgE glycoforms can be recognized by εBP.

IgE-binding protein (εBP)[1] (Liu, 1990) refers to a M_r 31 000 protein with IgE-binding activity, originally identified in rat basophilic leukemia (RBL) cells (Liu & Orida, 1984; Liu et al., 1985). Cloning and sequencing of cDNA revealed a novel sequence with several interesting structural features (Liu et al., 1985; Albrandt et al., 1987). The protein is composed of 2 domains: the amino-terminal domain contains tandem repeats of a highly conserved sequence of 9 amino acids [Tyr-Pro-Gly-(Pro/Gln)-(Ala/Thr)-(Pro/Ala)-Pro-Gly-Ala]; the

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‡ The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02921.
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[1] Abbreviations: εBP, IgE-binding protein; RBL, rat basophilic leukemia; CBP 35, carbohydrate-binding protein 35; RL-29, rat lung lectin 29; HL-29, human lung lectin 29; hnRNP, heterogeneous nuclear ribonucleoprotein; Mac-2, murine macrophage cell-surface protein 2.

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EXHIBIT B

carboxyl-terminal domain contains 21 of the 22 basic amino acids found in this protein (Albrandt et al., 1987). A subsequent study revealed that the protein has wide tissue distribution (Gritzmacher et al., 1988), suggesting that its function may not be limited to recognition of IgE. The primary cellular localization of this protein within the cytoplasm has also been demonstrated (Gritzmacher et al., 1988).

Although εBP contains interesting structral features, the function of this protein remains undefined. A recent development shed significant light on our understanding of the biochemical properties of εBP and provided a basis for probing physiological roles of this protein: another group of researchers, working with a carbohydrate-binding protein, CBP 35 (Roff & Wang, 1983), cloned cDNA coding for this protein (Jia & Wang, 1988) which revealed that it is highly homologous to εBP. In a collaborative effort, we showed that εBP, like CBP 35, has an intrinsic galactose-binding activity and that εBP and CBP 35 are rat and mouse protein homologues (Laing et al., 1989). Subsequently, it became clear that two other groups have been working with a similar lectin. One group has designated the lectin L-34, and the deduced protein sequence from their cDNA showed significant sequence homology with rat εBP (Raz et al., 1989). Another group has designated the lectin RL-29 (Leffler & Barondes, 1986) and HL-29 (Sparrow et al., 1987), from rat and human, respectively, and also has sequence data indicative of a close relationship with εBP (Leffler et al., 1989). It is now clear that εBP is an endogenous soluble lectin (Barondes, 1988) with S-type carbohydrate-recognition activity (Drickamer, 1988).

The exact physiological role(s) of εBP is (are) presently unknown, although studies of similar lectins in other laboratories have revealed interesting characteristics. CBP 35 can be found in the nucleus and may be a component of the heterogeneous nuclear ribonucleoprotein complex (hnRNP) (Laing & Wang, 1988); the overall level of CBP 35, as well as the amount of this protein in the nucleus, has been found to increase dramatically in proliferating 3T3 fibroblasts (Moutsatsos et al., 1987; Agrwal et al., 1989). These results suggest that this lectin may be a component of a growth-regulating system. Studies by Raz and co-workers related the expression of L-34 on the cell surface to neoplastic transformation and to the metastatic potential of tumor cells (Raz & Lotan, 1987). Recently, cDNA coding for the Mac-2 antigen, a murine macrophage cell-surface protein, has been shown to be identical with CBP 35, and thus a homologue of rat εBP (Cherayil et al., 1989). Several possible biological functions of this protein in macrophages, including a likely role as a cell-surface receptor involved in lectin-mediated phagocytosis, may provide a clue to the function of this protein in other biological systems.

In order to extend the study of this lectin to the human system, we decided to clone cDNA coding for the human counterpart of εBP. Here, we report the nucleotide sequence of human εBP cDNA. Comparison of the deduced amino acid sequence with that of the rat and murine sequences indicates that the proteins are highly conserved. Northern blot analysis identified a 1.1-kb mRNA similar in size to the previously characterized mRNA for rat εBP. Further, we show that εBP expression is cell-type-specific among various lymphoid cell lines. Protein of M_r 30000 could be isolated from several εBP-positive human cell lines with lactosyl-Sepharose and murine IgE–Sepharose and eluted specifically with lactose. In addition, an interesting pattern of εBP reactivity to human myeloma IgE was found, suggesting that only specific IgE glycoforms are recognized by human εBP.

Materials and Methods

Cell Lines and Reagents. The following cell lines were employed for this study: human B-lymphocyte cell line Wil-2 (from J. Marcelletti of this institute), human B-cell line RPMI 8866 (from D. Katz of this institute), human monocyte-like cell line U937 (American Type Cell Culture, CRL1593, Rockville, MD), rat basophilic leukemia (RBL) cell line (from H. Metzger, National Institutes of Health, Bethesda, MD), and Swiss 3T3 fibroblasts (American Type Cell Culture CCL92). Murine or human IgE–Sepharose 4B was prepared by using CNBr-activated Sepharose 4B (Pharmacia, Piscataway, NJ) and murine monoclonal anti-DNP IgE (Liu et al., 1980) or human myeloma IgE PS (from K. Ishizaka, La Jolla Institute of Allergy and Immunology, La Jolla, CA); DZA and BEDORA (from H. Spiegelberg, Scripps Clinic, La Jolla, CA) were prepared by following the manufacturer's protocol. Lactosyl-Sepharose 4B was prepared by derivatizing Sepharose 4B with lactose using a previously reported procedure (Levi & Teichberg, 1981). Rabbit antiserum directed to a synthetic peptide (FNPRFNENNRRVIVC), designated anti-εBP1, was employed in immunoblotting studies as described previously (Gritzmacher et al., 1988).

Screening cDNA Clones. cDNA clones were isolated from either a normal human lung λgt11 cDNA library (Clontech, Palo Alto, CA) or a HeLa cell library containing cDNA inserts in the λ Zap II vector (Stratagene, La Jolla, CA). Both libraries contain cDNA inserts cloned into the *Eco*RI site. The human lung λgt11 cDNA library (9×10^5 plaques) was screened by colony hybridization (Hanahan & Meselson, 1980) using nick-translated cDNA probes derived from the rat εBP cDNA clone E8 (Albrandt et al., 1987) and consisting of a 370 bp *Xho*I–*Sph*I amino-terminal cDNA fragment and a 378 bp *Sph*I–*Hae*III carboxy-terminal cDNA fragment. After identification of positive plaques, DNA from individual λgt11 clones was prepared followed by *Eco*RI digestion, isolation of the εBP insert, and subcloning into pUC19. One of the clones, 27.1, is described in this report.

The HeLa cell λ Zap II library (5×10^5 plaques) was initially screened with a ^{32}P-end-labeled 30-mer oligonucleotide probe (5′-AGGCCATCCTGGAGGGTTTGGGTTTCC-AGA-3′; Genetic Designs Inc., Houston, TX) representing the reverse complement of a sequence near the 5′ end of the human εBP-coding region (and corresponding to amino acids 14–23 of rat εBP) determined from a cDNA clone (27.1) identified in the human lung cDNA library described above. Hybridization was performed as described previously (Liu et al., 1988), and 67 positive plaques were identified. Selected plaques were then treated with R408 helper-phage to excise the pBluescript SK(−) phagemid following the manufacturer's protocol. By subsequent restriction endonuclease analysis, four clones containing the longest cDNA inserts were selected for DNA sequencing, and one clone (2.2) is included in this report.

Nucleotide Sequencing. Nested deletion subclones were produced for all cDNA clones by treatment of the plasmid DNA with *Exo*III–mung bean nuclease, ligation, and transformation, following the manufacturer's protocol (Stratagene). DNA was sequenced on both strands by the dideoxy chain termination method (Sanger et al., 1977) using modified phage T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH).

In Vitro Transcription and Translation. The clone 2.2 cDNA was digested with *Pvu*II, which produced a 1387 bp fragment containing the entire cDNA sequence and the T7 RNA polymerase promoter. The DNA was used to synthesize capped mRNA in vitro using T7 RNA polymerase (Strata-

gene) and a commercial transcription kit and protocol (Promega Corp., Madison, WI). The mRNA was then translated in vitro using [^{35}S]methionine in a rabbit reticulocyte lysate system (Promega). The εBP translation product was affinity-purified and analyzed as described below.

RNA Blot Analysis. Isolation of poly(A+) RNA from various cell lines, formaldehyde–agarose gel electrophoresis, and subsequent blotting to nitrocellulose filters were performed by using standard procedures (Maniatis et al., 1982). Hybridization was performed at 42 °C in 50% formamide/0.04% Ficoll/0.04% poly(vinylpyrrolidone)/0.04% BSA/75 mM NaCl/25 mM PIPES (pH 6.8)/25 mM EDTA/0.2% SDS/100 μg mL^{-1} tRNA/100 μg mL^{-1} denatured salmon sperm DNA using a nick-translated cDNA probe consisting of a 560 bp εBP fragment (derived from nested deletion of clone 27.1 and corresponding to clone 2.2 sequence spanning nucleotides 203–762) in pUC19. A final blot wash was performed at 37 °C in 0.1% SDS/30 mM NaCl/3 mM sodium citrate, pH 7.0, prior to autoradiography.

Immunoblot Analysis. Cell lysates were prepared by using Triton X-100 following a previously described procedure (Liu & Orida, 1984). Affinity purification was performed by mixing cell lysate supernatant with murine IgE–Sepharose 4B, human myeloma IgE–Sepharose 4B, or lactosyl-Sepharose 4B for 3 h at 4 °C followed by extensive washing with 1% Triton X-100/10 mM Tris-HCl (pH 7.5)/5 mM EDTA/150 mM NaCl followed by 62.5 mM Tris-HCl, pH 6.8. Bound proteins were eluted with either 0.2 M lactose or 2% SDS, fractionated by SDS–PAGE (Laemmli, 1970), transferred to filters (Immobilon, Millipore, Bedford, MA), and immunoblotted with a polyclonal anti-peptide antibody (anti-εBP1) as described (Gritzmacher et al., 1988).

Neuraminidase Treatment of Human Myeloma IgE–Sepharose and Reactivity with Rat ^{125}I-εBP. Human myeloma IgE (PS, DZA, or BEDORA)–Sepharose beads were suspended in PBS and treated with 0.5 unit of neuraminidase (Sigma, St. Louis, MO) for 1 h at 4 °C. Subsequently, the beads were washed several times with PBS and then used for immunoblot experiments or in a binding assay using radioiodinated recombinant rat εBP (L. Frigeri, M.W.R., and F.-T. L., unpublished results). The latter experiment utilized 25 μL of neuraminidase-treated beads suspended in 400 μL of 1% Triton-X100/10 mM Tris-HCl pH 7.5)/5 mM EDTA/150 mM NaCl and ^{125}I recombinant rat εBP labeled by the Chloramine T method (McDonahey & Dixon, 1966). After incubation for 3 h at 4 °C, the beads were washed extensively as described above, and bound protein was eluted with 50-μL portions of 0.2 M lactose. Aliquots of the pooled lactose eluate were characterized by SDS–PAGE followed by autoradiography.

Results

Cloning of Human εBP cDNA. As we had previously found that rat εBP is abundantly expressed in the lung, we first screened a normal human lung cDNA library with a rat εBP cDNA (Albrandt et al., 1987) probe and identified several clones. The clone (27.1) with the longest insert (2474 bp) was completely sequenced. This sequence was found to be highly homologous to rat εBP cDNA, with the following exceptions: (i) there is a much longer 5′-untranslated sequence in clone 27.1; (ii) there is a single base insertion in the coding region of clone 27.1, resulting in an interruption of the open reading frame. A computer data base search then revealed that a part of the "5′-untranslated sequence" matches closely with the coding region of the ferritin gene. We thus concluded that clone 27.1 is a fused cDNA that also has a single base insertion, probably results of cloning artifacts. Several other clones were found to contain partial εBP coding sequence. Significantly, each of these clones did not have the single base mutation noted in clone 27.1.

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CCAGCCAACGAGCCGGAAA ATG GCA GAC AAT TTT TCG CTC CAT GAT GCG TTA      51
                    M   A   D  (N) F   S   L   H   D   A   L
                                                        10
TCT GGG TCT GGA AAC CCA AAC CCT CAA GGA TGG CCT GGC GCA TGG GGG      99
 S   G   S   G   N   P   N   P   Q   G   W   P   G   A   W   G
                                20
AAC CAG CCT GCT GGG GCA GGG GGC TAC CCA GGG GCT TCC TAT CCT GGG     147
 N   Q   P   A   G   A   G   G   Y   P   G   A   S   Y   P   G
         30                                          40
GCC TAC CCC GGG CAG GCA CCC CCA GGG GCT TAT CCT GGA CAG GCA CCT     195
 A   Y   P   G   Q   A   P   P   G   A   Y   P   G   Q   A   P
                        50
CCA GGC GCC TAC CAT GGA GCA CCT GGA GCT TAT CCC GGA GCA CCT GCA     243
 P   G   A   Y   H   G   A   P   G   A   Y   P   G   A   P   A
 60                                          70
CCT GGA GTC TAC CCA GGG CCA CCC AGC GGC CCT GGG GCC TAC CCA TCT     291
 P   G   V   Y   P   G   P   P   S   G   P   G   A   Y   P   S
             00                                          90
TCT GGA CAG CCA AGT GCC CCC GGA GCC TAC CCT GCC ACT GGC CCC TAT     339
 S   G   Q   P   S   A   P   G   A   Y   P   A   T   G   P   Y
                            100
GGC GCC CCT GCT GGG CCA CTG ATT GTG CCT TAT AAC CTG CCT TTG CCT     387
 G   A   P   A   G   P   L   I   V   P   Y   N   L   P   L   P
         110                                         120
GGG GGA GTG GTG CCT CGC ATG CTG ATA ACA ATT CTG GGC ACG GTG AAG     435
 G   G   V   V   P   R   M   L   I   T   I   L   G   T   V   K
                        130
CCC AAT GCA AAC AGA ATT GCT TTA GAT TTC CAA AGA GGG AAT GAT GTT     483
 P   N   A   N   R   I   A   L   D   F   Q   R   G   N   D   V
140                                         150
GGC TTC CAC TTT AAC CCA CGC TTC AAT GAG AAC AAC AGG AGA GTC ATT     531
 A   F   H   F   N   P   R   F   N   E   N   N   R   R   V   I
             160                                         170
GTT TGC AAT ACA AAC CTG GAT AAT AAC TGG GGA AGG GAA GAA AGA CAG     579
 V   C   N   T   K   L   D   N   N   W   G   R   E   E   R   Q
                                100
TCG GTT TTC CCA TTT GAA AGT GGG AAA CCA TTC AAA ATA CAA GTA CTG     627
 S   V   F   P   F   E   S   G   K   P   F   K   I   Q   V   L
         190                                         200
GTT GAA CCT GAC CAC TTC AAG GTT GCA GTG AAT GAT GCT CAC TTG TTG     675
 V   E   P   D   H   F   K   V   A   V   N   D   A   H   L   L
                        210
CAG TAC AAT CAT CGG GTT AAA AAA CTC AAT GAA ATC AGC AAA CTG GGA     723
 Q   Y   N   H   R   V   K   K   L   N   E   I   S   K   L   G
220                                         230
ATT TCT GGT GAC ATA GAC CTC ACC AGT GCT TCA TAT ACC ATG ATA TAA     771
 I   S   G   D   I   D   L   T   S   A   S   Y   T   M   I  ✡
             240                                         250
TCTGAAAGGGGCAGATTAAAAAAAAAAAAAAAAGAATCTAAACCTTACATGTGTAAAGGTTTCA   834
TGTTCACTGTGAGTGAAAATTTTTACATTCATCAATATCCCTCTTGTAAGTCATCTACTTAAT    897
AAATATTACAGTGAAAG                                                  914
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FIGURE 1: Nucleotide sequence of human εBP 2.2 cDNA and its deduced amino acid sequence. The deoxynucleotide numbering scheme is shown on the right margin, and the amino acid sequence is numbered below each line. The polyadenylation signal is underlined. The potential N-linked glycosylation site (*Asn*XaaSer/Thr) is circled. The identity between clone 27.1 and clone 2.2 begins at nucleotide 36 and amino acid 7 (leucine) in clone 2.2. The single nucleotide addition (dC) in clone 27.1 appears between nucleotides 500 and 501 in clone 2.2. In addition, nucleotide 286 (dC) in clone 2.2 is different from the corresponding position in clone 27.1 (dT), resulting in a proline (2.2) to serine (27.1) substitution.

A survey of several cell lines revealed that HeLa cells express an abundant level of εBP protein, and therefore we explored a cDNA library derived from HeLa cell mRNA in order t obtain a full-length cDNA clone. Several clones were again identified, and partial sequencing revealed they were siblings containing inserts of nearly equal length. One of the clones (2.2) was completely sequenced and is reported in Figure 1. The cloned cDNA contains 18 bp of 5′-untranslated sequence which was unique compared to the 5′-noncoding sequence of clone 27.1, 750 bp of coding region, and 146 bp of 3′-untranslated sequence. A typical polyadenylylation signal (AATAAA) is found 14 bp from the 3′-terminus of the cDNA.

In Vitro Transcription and Translation of the Cloned cDNA. In order to demonstrate that cDNA clone 2.2 codes for a protein with the expected properties of εBP (i.e., lactose-binding activity), mRNA was transcribed from the cloned cDNA and then translated in vitro. The translation products

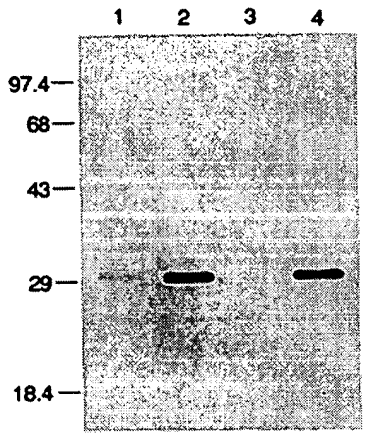

FIGURE 2: In vitro transcription and translation of cDNA clone 2.2 followed by fractionation on lactosyl-Sepharose. Clone 2.2 was transcribed and translated in a cell-free system using [^{35}S]methionine as label. Thereafter, translation products were incubated with either BSA–Sepharose followed by elution with 2% SDS (lane 1) or with lactosyl-Sepharose followed by elution with 2% SDS (lane 2), 0.2 M glucose (lane 3), or 0.2 M lactose (lane 4). Eluted proteins were analyzed by SDS–PAGE (10% polyacrylamide) and fluorography. The numbers on the left represent molecular weight ($\times 10^{-3}$) markers.

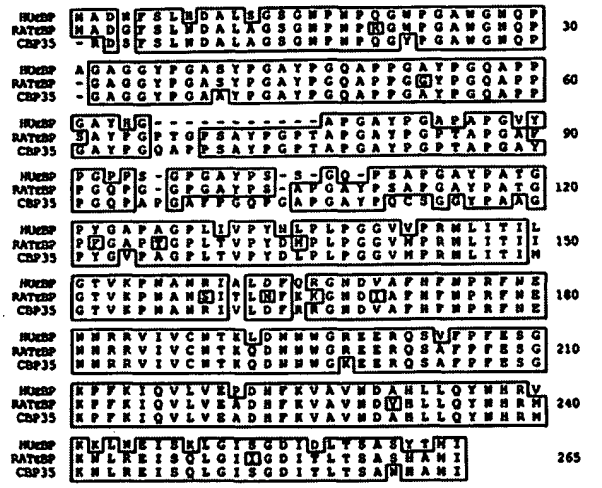

FIGURE 3: Comparison of amino acid sequences between human, mouse (CBP 35), and rat εBP. The sequences were positioned for best fit using the IALIGN program, and the single-letter amino acid code is used. Boxes indicate sequence identity, and numbers on the right margin indicate amino acid position.

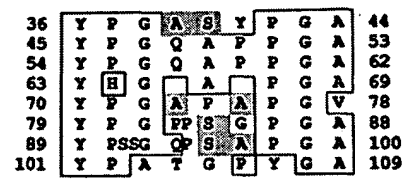

FIGURE 4: Human εBP contains a highly conserved repeated sequence in the amino-terminal domain. A nine amino acid segment is repeated 8 times.

were absorbed with lactosyl-Sepharose, previously shown to effectively bind rat εBP, and subsequently eluted with buffers containing either SDS or lactose. As shown in Figure 2, a protein of M_r 30000 was detected by SDS–PAGE using either SDS (lane 2) or lactose (lane 4) elution but not in glucose-eluted (lane 3) samples. In addition, the protein did not bind significantly to BSA–Sepharose 4B (lane 1), indicating that, by comparison, the binding to lactosyl-Sepharose is specific. These results confirm that the cloned sequence indeed codes for a protein with lactose-binding activity.

High Conservation of εBP Protein Sequence. The amino acid sequence deduced from cDNA clone 2.2 is included in Figure 1. The sequence of human εBP, encoding a protein with a predicted molecular weight of 26.2K, does not contain a recognizable signal sequence nor a sequence characteristic of a transmembrane domain, and, as such, is similar to mouse and rat εBP. However, unlike the mouse and rat sequence, the predicted human sequence contains a potential N-linked glycosylation site (*Asn*XaaSer/Thr) at Asn^3. Whether this site is utilized or not has not been determined, but by SDS–PAGE and gel to gel comparison of in vitro translated εBP (nonglycosylated) to εBP isolated from either Wil-2 or HeLa cells, it appears that the apparent molecular weights are very similar, suggesting the potential N-linked glycosylation site is not utilized.

The human εBP sequence was compared to the corresponding rat (Albrandt et al., 1987) and murine (Jia & Wang, 1988) sequences using the IALIGN program, as shown in Figure 3. Of the 250 amino acids, 209 residues (84%) are identical with rat εBP, 205 residues (82%) are identical with murine εBP (CBP 35), and 220 residues (88%) are identical with either rat or murine εBP. The most noticeable difference found among the sequences is the absence of a 11-residue stretch in the human sequence within the amino-terminal portion. Significantly, this "deletion" was found in separate clones isolated from different libraries (normal human lung and HeLa cell), suggesting that it is neither a cloning artifact nor an isolated mutational event in the cells from which the original mRNA was derived.

Similar to rat and murine εBP, the human εBP sequence can be divided into two domains. The amino-terminal domain is composed of highly conserved repeated sequences as shown in Figure 4. Due to the "deletion" of 11 residues mentioned above and 2 other gaps in the human sequence, as compared to rat εBP, there are only eight repeats compared to the 10 repeats noted for rat εBP. The consensus sequence, Tyr-Pro-Gly-(Gln/Ala)-(Ala/Ser)-(Pro/Ala)-Pro-Gly-Ala, is also slightly different from that of rat εBP with the most notable difference found at the fifth amino acid position (consensus Ala/Ser in human εBP compared to Ala/Thr in rat εBP). The carboxyl-terminal domain, on the other hand, is highly conserved. Particularly striking is the region between Arg^{166} and Arg^{239} where only 6 differences are found in the 74-residue segment (92% identity) between human and rat εBP and only 4 differences (95% identity) between human εBP and CBP 35. Moreover, within this region, all residues are shared by at least two of the species compared. Finally, it should be pointed out that both rat and human εBP have a single cysteine residue while mouse εBP (CBP 35) has a second in addition to the conserved site.

Cell Type Specific Expression of Human εBP mRNA. A Northern blot analysis was performed using the cloned human εBP cDNA to probe RNA isolated from HeLa cells. As shown in Figure 5, lane 2, a predominant hybridizing band of 1.1 kb was detected which is similar in size to that previously reported for rat εBP mRNA (Liu et al., 1985) (see lane 1).

Previously, we demonstrated the expression of εBP in a variety of tissues and different cell lines. With the human εBP clone in hand, we did an initial survey of selected human cell lines for the presence of εBP mRNA. As shown in Figure 5, a spectrum of εBP mRNA levels was detected: a human B-cell line (Wil-2) showed the highest level (lane 5) followed by a very low level in the monocyte line, U937 (lane 3), and no detectable εBP mRNA in another B-cell line, RPMI 8866

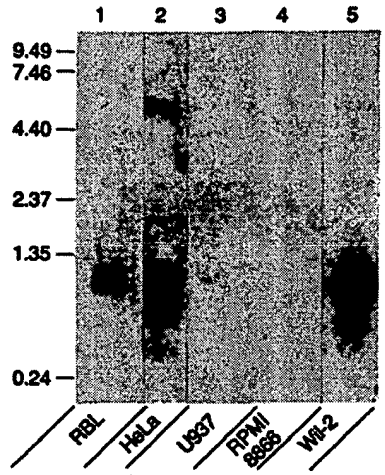

FIGURE 5: Northern blot of mRNA derived from various cell lines. Poly(A+) RNA was electrophoresed on a 1% agarose–formaldehyde gel, blotted, and probed with a human εBP cDNA fragment spanning the εBP coding region. The following mRNAs were probed: RBL (5 μg), lane 1; HeLa (2.5 μg), lane 2; U937 (5 μg), lane 3; RPMI 8866 (5 μg), lane 4; Wil-2 (5 μg), lane 5. Numbers on the left margin represent RNA molecular weight ($\times 10^{-3}$) markers.

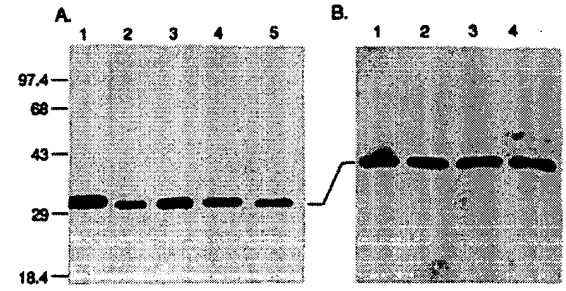

FIGURE 6: Immunoblot of human εBP isolated from HeLa cells or Wil-2 cells and affinity-purified using lactosyl-Sepharose or murine IgE–Sepharose. (Panel A) HeLa cell protein extracts were affinity-purified (i) using lactosyl-Sepharose 4B with bound protein subsequently eluted with SDS–PAGE sample buffer (lane 2) or 0.2 M lactose (lane 3) or (ii) using murine IgE–Sepharose 4B and elution with SDS (lane 4) or 0.2 M lactose (lane 5). Lane 1 is rat εBP derived from RBL cells, affinity-purified with lactosyl-Sepharose 4B, and eluted with SDS. (Panel B) Wil-2 protein extracts were affinity-purified using murine monoclonal anti-DNP IgE–Sepharose 4B (lane 1, SDS elution; lane 2, 0.2 M lactose elution) or lactosyl-Sepharose 4B (lane 3, SDS elution; lane 4, 0.2 M lactose elution). The isolated proteins were analyzed by SDS–PAGE (10% in panel A, 12.5% in panel B) followed by immunoblotting. Numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers.

(lane 4). When clone 27.1 cDNA (which contains a partial ferritin gene sequence, as mentioned above) was used, an intense hybridizing band at ~1.0 kb (which we assume to be ferritin mRNA) was detected in the RNA blot of RPMI 8866 and U937 RNA, supporting the integrity of the RNA preparation (data not shown). Additional RNA blotting experiments revealed either very low or nondetectable levels of εBP in the DAUDI human B cell line and the HUT78 human T-cell line, respectively (data not shown).

Isolation of Human εBP Protein. We next performed experiments to show the existence of human εBP protein in cells. A monospecific antibody, previously generated against a peptide sequence in rat εBP (Gritzmacher et al., 1988) (now known to be identical in human εBP), was a particularly useful probe for immunoblotting studies. Protein extracts from HeLa cells were subjected to affinty purification with murine IgE–Sepharose 4B or lactosyl-Sepharose 4B, and eluted protein was analyzed by SDS–PAGE followed by immunoblotting using

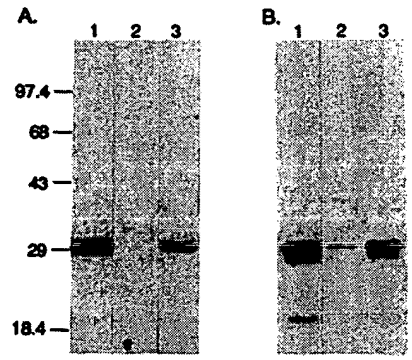

FIGURE 7: Recognition of εBP by human and murine IgE–Sepharose 4B. (Panel A) Immunoblot of εBP, derived from HeLa extracts, bound to either neuraminidase-treated murine IgE–Sepharose 4B (lane 1), human myeloma IgE(PS)–Sepharose 4B (lane 3), or untreated IgE(PS)–Sepharose 4B (lane 2) followed by 0.2 M lactose elution. (Panel B) Immunoreactivity of ^{125}I-labeled recombinant rat εBP with neuraminidase-treated murine IgE–Sepharose 4B (lane 1), human IgE(PS)–Sepharose 4B (lane 3), or untreated IgE(PS)–Sepharose 4B (lane 2) followed by 0.2 M lactose elution. Both panels show protein resolved by 10% SDS–PAGE followed by either immunoblotting (panel A) or autoradiography (panel B). Numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers.

the sequence-specific antibody. As shown in Figure 6 (panel A), a protein band of M_r 30 000 was detected from both adsorbents eluted with SDS or lactose (lanes 2–5). By comparison with rat εBP (lane 1), human εBP appears to migrate slightly faster, perhaps due to the 11-residue "deletion" found for human εBP. The results also suggest either that the potential glycosylation site predicted in human εBP is not utilized or that the glycosylation does not significantly increase the apparent molecular weight of human εBP. The combination of using specific ligand (IgE–Sepharose and lactosyl-Sepharose) and sequence-specific antibodies, together with the molecular size, is highly supportive that the detected protein is human εBP.

Protein extracts from Wil-2 cells were similarly analyzed, using murine IgE–Sepharose in addition to lactosyl-Sepharose. As shown in Figure 6 (panel B), εBP bound essentially equivalently to either murine IgE–Sepharose 4B (lanes 1 and 2) or lactosyl-Sepharose 4B (lanes 3 and 4) and was eluted by either SDS (lanes 1 and 3) or lactose (lanes 2 and 4) from both affinity supports. These results further establish the carbohydrate-binding activity of this protein and also cross-species reactivity of human εBP with murine IgE.

Recognition of Different Human IgE Glycoforms by Human εBP. Having established reactivity of human εBP to both lactosyl-Sepharose 4B and murine IgE–Sepharose 4B, we then investigated reactivity to myeloma human IgE(PS)–Sepharose 4B. Surprisingly, human εBP derived from HeLa extracts did not bind to the immobilized myeloma IgE(PS) as shown by immunoblot analysis (Figure 7, panel A, lane 2). A similar lack of reactivity was found for two other human myeloma IgEs (DZA and BEDORA) with HeLa-derived εBP in addition to no discernible recognition of εBP, produced from Wil-2 cells, by IgE(PS)–Sepharose (data not shown).

From a carbohydrate mapping study, it is known that myeloma IgE(PS) is extensively sialyated (Baenziger et al., 1974). Because sialic acid (*N*-acetylneuraminic acid) residues are typically found attached to terminal galactose residues in glycoproteins, we considered the possibility that lack of reactivity of myeloma IgE(PS) to εBP was due to the presence of sialic acid residues blocking presentation of the appropriate galactose-capped oligosaccharide determinants. Thus, myeloma IgE(PS)–Sepharose was treated with neuraminidase and

then evaluated for reactivity to εBP. As shown in Figure 7, panel A, the use of neuraminidase-treated human IgE(PS)–Sepharose (lane 3) resulted in a significant degree f recognition between human IgE and εBP. An identical pattern of reactivity was also found for two other human myeloma IgEs, DZA and BEDORA (data not shown). In a parallel experiment (Figure 7, panel B), ^{125}I-labeled recombinant rat εBP was shown to exhibit very slight reactivity to IgE(PS)–Sepharose (lane 2) but pronounced binding to neuraminidase-treated IgE(PS)–Sepharose (lane 3). The results indicate that desialylated human myeloma IgE exhibits recognition of εBP and that this recognition is largely blocked by sialylation of IgE carbohydrate chains.

Discussion

This paper reports the isolation from human HeLa cells of cDNA coding for human IgE-binding protein. The authenticity of the cloned cDNA is supported by the following observations: (i) mRNA transcribed from the cDNA can be translated in vitro to afford a protein similar in size to rat εBP and with lactose-binding activity; (ii) the deduced amino acid sequence showed extensive sequence homology to rat and murine εBP; (iii) like rat and murine εBP, the deduced protein sequence of human εBP is also composed of a two-domain structure with the amino-terminal domain containing tandem repeats of a highly conserved amino acid sequence; (iv) the carboxyl-terminal domain exhibited significant homology with consensus sequences from a family of S-type lectins; (v) the cloned cDNA hybridizes with mRNA from several human cell lines which was similar in size to rat εBP mRNA; (vi) a protein of M_r 30 000 was isolated from two human cell lines which is recognized by antibodies directed to a peptide sequence predicted in human εBP. In addition, we demonstrate that human εBP, like rat and murine εBP, binds to murine IgE–Sepharose and lactosyl-Sepharose and the binding can be reversed by lactose. We thus conclude that human εBP is also an endogenous lectin with β-galactoside-binding activity.

Several observations accrued during this study to support the contention that cDNA clone 2.2 contains the entire coding sequence for the mature protein: (i) the position of the translation initiation methionine (and adjacent coding sequence) for the rat (Albrandt et al., 1987) and murine (Raz et al., 1989; Cherayil et al., 1989) homologues aligns quite favorably with the start methionine proposed for clone 2.2; (ii) the apparent molecular weight of in vitro translated εBP, as determined by SDS–PAGE and gel to gel comparison, is very similar to that found for εBP derived from several human cell lines. As an extension of these observations, it is reasonable to infer that the cDNA sequence previously reported for rat εBP (Albrandt et al., 1987) also contains full-length coding sequence despite the small discrepancy reported for the apparent molecular weight of in vitro translated rat εBP compared to εBP isolated from RBL cells (Albrandt et al., 1987).

An interesting finding in this study is the significant conservation of the amino acid sequence among the three animal species compared. This pertains to both the amino-terminal and carboxyl-terminal domains, suggesting both parts are important for the function of this protein. The amino-terminal domain exhibits sequence similarity to protein components of hnRNP; in fact, it has been demonstrated that CBP 35 (equivalent to εBP) forms complexes with nuclear RNA (Laing & Wang, 1988). By FASTP analysis, a generally similar degree of homology (20% identity) was found between human εBP and the hnRNP sequences surveyed by Wang et al. (Jia & Wang, 1988). Whether this homology reflects an evolutionary relationship between these proteins is not known.

Comparison of the sequence in the carboxyl-terminal domain of εBP among the three species is quite informative. The region between Arg^{166} and Arg^{239} is essentially completely conserved as detailed under Results. This area has been previously noted (Liu, 1990; Jia & Wang, 1988) to possess significant homology between εBP/CBP 35 and another group of S-type soluble lectins of M_r 14K–16K (Clerch et al., 1988; Raz et al., 1988; Southan et al., 1987; Gitt & Barondes, 1986; Paroutaud et al., 1987). In particular, one stretch of amino acids (HFNPRF, amino acids 173–178, human εBP) is invariant for εBP derived from the three species and identical with a consensus sequence of the 14–16-kDa lectins (Levi & Teichberg, 1981). An additional human εBP sequence (*W*GX*E*ERQ; amino acids 196–202) is also highly conserved compared to the rat and murine sequences and exhibits conserved Trp and Glu residues (italicized) previously implicated as critical sites for lectin activity (Levi & Teichberg, 1981). Together these comparisons indicate a high level of conservation in regions of putative carbohydrate-binding activity which may, in turn, indicate some relationship between these lectins in specificity and/or function.

Another interesting finding is the cell-specific expression of εBP in various lymphoid cell lines. It appears that expression of εBP may be dependent on lymphocyte differentiation, as the three cell lines tested express varying cell-surface markers that represent different stages of B-cell differentiation (for Wil-2, FcϵR$^+$, FcγR$^+$, Ig nonproducer; for RPMI-8866, FcϵR$^+$, FcγR$^-$, IgG producer; for DAUDI, FcϵR$^-$, FcγR$^+$, Ig nonproducer). The expression of CBP 35 has been shown t be developmentally regulated in that the protein is abundant in certain embryonic tissues but low in corresponding tissues in the adult (Crittenden et al., 1984). In addition, the role of lectins and glycoconjugates in cell differentiation has been well documented (Feizi, 1985; Sharon & Lis, 1989). Therefore, differentiation-dependent expression of εBP is certainly possible. Significantly, expression of the Mac-2 antigen, which is identical with CBP 35 (Cherayil et al., 1989), has been shown to increase during maturation of macrophage precursors (Leenen et al., 1986) which further supports a link between this protein and cell development. Further investigation will be necessary to establish a correlation between εBP expression and lymphocyte differentiation.

The initial characterization of protein corresonding to human εBP resulted in the demonstration of both carbohydrate-binding activity and cross-species reactivity to murine IgE. Both results parallel the reactivity pattern shown by the murine (CBP 35) and rat homologues and are consistent with the highly conserved interspecies amino acid sequence, particularly within the carboxy terminus, of these proteins. Perhaps the most interesting finding in this study, however, was the pattern of reactivity found between εBP and human IgE. The lack of binding of human εBP to three different human myeloma IgE proteins was, initially, quite surprising. However, it did provide a clue to the existence of carbohydrate heterogeneity among IgE proteins. Since human εBP does bind murine IgE as well as neuraminidase-treated human myeloma IgE, we concluded that the lack of significant reactivity of human εBP to untreated myeloma IgE is most likely due to the masking of the εBP ligand in IgE, i.e., galactosides, by sialylation. In fact, it has been shown by Sparrow et al. (1987) that HL-29 (which is likely the equivalent of human εBP) binds lactose to a much greater extent than lactose derivatized with sialic acid (6-sialolactose).

One possible explanation for the observed εBP selectivity in IgE recognition is that oligosaccharide chains present on

human IgE are significantly different compared to murine IgE and this difference precludes recognition of human IgE by εBP. This phenomenon may indicate that there is a species-dependent pattern f reactivity between IgE and εBP. Alternatively, the myeloma IgEs tested may not be representative of normal polyclonal IgE, particularly with regard to structure and composition of carbohydrates. Indeed, it has been documented that proteins from transformed cells, including in vivo tumors, often exhibit different carbohydrate composition and structure relative to nontransformed cells (Hakomori, 1984). Moreover, this difference has been shown to take the form of excessive sialylation of O-linked oligosaccharides in some examples (Hakomori, 1984). In either event, it should be informative to evaluate εBP reactivity to samples of polyclonal IgE derived from nontransformed cells.

The significance of IgE-binding activity for εBP remains an interesting question. Since demonstration of the lectin property for εBP, it became clear that IgE binding may be accounted for by the recognition of carbohydrates on IgE. In addition, this recognition has been reported to show isotype specificity (Cherayil et al., 1989) which is in agreement with our own findings (unpublished results). Therefore, with the demonstrated recognition of murine IgE and neuraminidase-treated human IgE by εBP, a possible function in the IgE system cannot be excluded. It is interesting to contrast εBP to the low affinity IgE receptor (FcεRII) which also contains a lectin domain (Kikutani et al., 1986; Ikuta et al., 1987; Ludin et al., 1987). One significant difference between the two is that FcεRII appears to recognize IgE protein sequence and its binding to IgE apparently is not carbohydrate dependent (Vercelli et al., 1989). However, FcεRII has been shown to bind carbohydrates (Richards & Katz, 1990), and its functions may not be limited to those mediated through recognition of IgE. A particularly attractive possibility for εBP function in the IgE system may be a role in augmenting IgE-dependent mast cell activation. Recent demonstration that the Mac-2 antigen (equivalent to CBP35/εBP) is found, in part, to be secreted into the extracellular space (Cherayil et al., 1989) suggests that, under the appropriate stimulus, εBP may respond similarly. In the event the εBP could be secreted, it is conceivable that, through IgE binding, it might activate IgE-bound mast cells, resulting in degranulation in an antigen-independent manner. These possibilities await future investigation.

The physiological role(s) of εBP (CBP 35, L-34, RL-29, and Mac-2) is (are) intriguing. So far, studies by other investigators have related this lectin to growth regulation, cell transformation, metastasis, and, in macrophages, inflammatory signals. The detection of this lectin in the cytoplasm, nucleus, and cell surface suggests it may have multiple functions. Isolation of human εBP cDNA reported herein established the existence of the human counterpart of εBP and revealed a high conservation of protein sequence. Detection of a spectrum of εBP mRNA levels from several B-lymphocyte cell lines was found and may reflect a link between lymphocyte differentiation and εBP expression. The cDNA described herein should facilitate the analysis of both gene expression of this protein in various physiological and pathological conditions in human systems and structure–function correlates of εBP, thereby improving our understanding of the function(s) of this lectin.

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Protection from Photoinhibition by Low Temperature in *Synechocystis* 6714 and in *Chlamydomonas reinhardtii*: Detection of an Intermediary State

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ABSTRACT: Photoinhibition was induced in a cyanobacterium strain, *Synechocystis* 6714, and a green alga, *Chlamydomonas reinhardtii*, by exposing them to light intensities from 1000 to 4000 $\mu E/(m^2 \cdot s)$ at various temperatures. The photoinhibition process was followed by measurements of chlorophyll fluorescence and oxygen evolution. During exposure to high light, fluorescent active reaction centers II became low fluorescent inactive centers. This process involved several reversible and irreversible steps. The pathway from the active state to the inactive low fluorescent state was different in *Synechocystis* and *Chlamydomonas*. In the latter there was a reversible intermediary step characterized by an increase of F_o. This state was stable at 5 °C and slowly reversible at room temperature. The high F_o fluorescence level corresponded to a state of photosystem II centers that were inactive for oxygen evolution. An F_0 decrease occurred in the dark in the absence of protein synthesis and was correlated to a restoration of oxygen evolution. Further experiments suggested that the existence of the intermediate fluorescent state is due to modified closed centers in which the reduced primary acceptor is less accessible to reoxidation. In cyanobacteria this reversible state was not detected. In both organisms, the decrease of F_{max} reflected an irreversible damage of photosystem II centers. These centers need replacement of proteins in order to be active again. The quenching of F_{max} and the irreversible inhibition of oxygen evolution were slowed down in both organisms by decreasing the temperature of the photoinhibitory treatment from 34 to 5 °C. We conclude that low temperature protected the reaction center II from irreversible photodamage.

Photoinhibition is related to the excess of light absorbed by the pigment antennae which cannot be properly dissipated by photosynthesis (Osmond, 1981; Powles, 1984; Kyle, 1987; Cleland, 1988). There is now a large amount of evidence demonstrating that the primary site of lesion is the reaction center of the photosystem II (PSII).[1] Different sites in the reaction center II (RCII) were proposed to be the first target of high light. Studies on isolated chloroplasts, thylakoids, or PSII preparations suggested that the P_{680}–Phe–Q_A portion of the electron transport is the primary site of damage (Cleland et al., 1986; Theg et al., 1986; Arntz & Trebst, 1986; Vass et al., 1988; Styring et al., 1990). As opposed to that, results obtained with intact organisms suggested that the Q_B niche in the D_1 protein is the first site to be damaged (Kyle et al., 1984; Kirilovsky et al., 1988; Ohad et al., 1988). It was observed that the electron transfer through Q_B ($H_2O \rightarrow$ DCBQ or $H_2O \rightarrow$ DCIP) decreased more rapidly than electron transfer which did not involve Q_B ($H_2O \rightarrow$ SiMo) (Kirilovsky et al., 1988, Kyle et al., 1984). Moreover, thermoluminescence measurements showed that modifications of the B band appeared before modifications of the Q band. B and Q signals result from the charge recombination between $S_{2,3}$ and Q_B^- and Q_A^-, respectively (Ohad et al., 1988; Kirilovsky, Ducruet, and Etienne, unpublished data).

The decrease of PSII activity due to photoinhibition can be restored if the cell exposure to high light is not too long. The repair process, which is light dependent, involves de novo synthesis of thylakoid proteins among which the most prominent is D_1 (Ohad et al., 1985; Lönneborg et al., 1988; Kirilovsky et al., 1988).

It is assumed that environmental conditions that reduced the rate of photosynthesis accentuate the effects produced by plants exposed to high light. Exposure of leaves of many plants to high photon flux densities at chilling temperatures produces a damage to the photosynthetic apparatus which is greater than that observed at higher temperatures [reviewed by Oquist et al. (1987)]. The recovery is also temperature dependent, being slower at low temperatures. It was proposed that the inhibition of recovery by low temperatures may also contribute to the particular susceptibility to photoinhibition in plants at chilling

[1] Abbreviations: Chl, chlorophyll; D_1 and D_2, polypeptides of the RCII; DCBQ, dichlorobenzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_o, F_v, F_{max}, initial, variable, and maximum fluorescence; P_{680}, a chlorophyll molecule that acts as the primary electron donor in the RCII; Pheo, pheophytin, intermediary electron acceptor; PSII, photosystem II; Q_A and Q_B, primary and secondary quinone electron acceptors, respectively; RCII, reaction center II; Z, electron donor to P_{680}.

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Immunology

Molecular cloning of a human macrophage lectin specific for galactose

(Mac-2 antigen/cDNA sequence/laminin binding)

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ABSTRACT **The murine Mac-2 protein is a galactose- and IgE-binding lectin secreted by inflammatory macrophages. We describe here the cloning and characterization of a cDNA representing the human homolog of Mac-2 (hMac-2). The amino acid sequence derived from the hMac-2 cDNA indicates that the protein is evolutionarily highly conserved, with 85% of its amino acid residues being similar to those in the murine homolog. This conservation is especially marked in the carboxyl-terminal lectin domain. The amino-terminal half of the protein is less conserved but still contains the repetitive proline-glycine-rich motif seen in the mouse protein. hMac-2 synthesized *in vitro* is recognized by the M3/38 monoclonal antibody to Mac-2 and binds to the desialylated glycoprotein asialofetuin and to laminin, a major component of basement membranes. These findings are discussed in the context of the potential functions of hMac-2.**

Macrophages activated during the course of acute or chronic inflammation release a number of soluble proteins which mediate or regulate the effects of the inflammatory response. Understanding the function and mode of regulation of these monokines is critical to devising strategies for therapeutic intervention in inflammatory disorders. The murine Mac-2 antigen was originally described by Ho and Springer (1) and was shown to be expressed at a high level on the surface of inflammatory macrophages. We recently cloned cDNAs encoding Mac-2 and showed that the protein is secreted and has the characteristics of a galactose-specific lectin (2). A search of the computer data bases revealed that it had been identified independently by two other groups on the basis of its carbohydrate-binding property. Wang and colleagues (3, 4) identified the protein, which they called carbohydrate-binding protein 35 (CBP 35), in 3T3 mouse fibroblasts, and on the basis of immunolocalization studies and sequence homologies proposed that it might be a component of nuclear ribonucleoprotein complexes. Raz *et al.* (5) identified the same protein (under the name L-34) as a tumor cell surface lectin and suggested that it enhanced tumor metastasis by promoting the formation of multicellular emboli. A rat cytosolic protein highly similar to Mac-2/CBP 35/L-34 has been described and has been shown to bind IgE (6). We have shown that Mac-2 also has the ability to bind murine IgE (2). Recently, yet another function has been revealed by the work of Woo *et al.* (7), who showed that Mac-2 is the major nonintegrin laminin-binding protein synthesized by murine inflammatory macrophages, indicating a potential role in macrophage–extracellular matrix interactions.

Given the number of functions and subcellular locations proposed for Mac-2, further studies are clearly warranted. The high expression of this protein in inflammatory macrophages suggests that it has an important function in inflammation. In view of the potential involvement of this protein in processes relevant to human disease, we were interested in determining whether a human homolog of Mac-2 existed. In the present report we describe the cloning of the human homolog of Mac-2 (which we have designated hMac-2, for human Mac-2 antigen)* and show that the primary structure of the two proteins is highly conserved, especially in the lectin domain. The hMac-2 synthesized *in vitro* is recognized by the M3/38 monoclonal antibody to Mac-2 (1) and behaves like a galactose-specific lectin in its binding to the desialylated glycoprotein asialofetuin. It also binds to purified laminin, thus confirming and extending the results of Woo *et al.* (7).

MATERIALS AND METHODS

A cDNA library made from *Staphylococcus albus*-activated human monocytes in the vector λgt11 (kindly provided by Deborah Galson, Massachusetts Institute of Technology) was screened with the radiolabeled 970-base-pair (bp) *Eco*RI insert from the clone Mac 2.16 (2) under the following conditions: hybridization at 65° in 6× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.2% SDS/1× Denhardt's solution (0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin)/1 mM EDTA and salmon sperm DNA at 100 μg/ml for 18 hr; wash with 3×SSC/0.1% SDS at 37°C. Positive plaques were purified, DNA was prepared, and the inserts were subcloned in the vector pBSK (Stratagene) as previously described (2).

To obtain 5′-extended hMac-2 cDNAs, the 660-bp *Eco*RI fragment from the clone hMac-2.2 was radiolabeled and used to screen, under high-stringency conditions (8), a cDNA library made from the human colonic carcinoma cell line HT29 in the vector CDM8 (9) (kindly provided by Ivan Stamenkovic, Massachusetts General Hospital). Positive colonies were purified, and plasmid DNA was prepared (8).

Double-stranded DNA sequencing was carried out with the T7 sequencing kit (Pharmacia), using appropriate restriction fragments cloned in pBSK. Oligonucleotide primers based on available sequence were used when the restriction fragment was too large to be sequenced by using the vector-based primers. Sequence comparisons were carried out with the computer program GAP (10).

In vitro transcripts of cloned cDNAs were generated by using the T7 promoter in CDM8 and the transcripts were translated *in vitro* as previously described (2). Northern analysis, Western analysis, metabolic labeling of cells, immunoprecipitations, precipitations with asialofetuin coupled to Sepharose, and SDS/PAGE were carried out as detailed earlier (2).

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Abbreviations: hMac-2, human Mac-2 antigen; PMA, phorbol 12-myristate 13-acetate.

*The sequence reported in this paper has been deposited in the GenBank data base (accession number M35368).

EXHIBIT C

Binding of [^{35}S]methionine-labeled *in-vitro*-synthesized hMac-2 protein to laminin was carried out as follows. One milligram of laminin purified from the Engelbreth–Holm–Swarm sarcoma (GIBCO) was coupled (2) to 1 ml of Sepharose CL-4B (Pharmacia) and stored as an approximately 50% slurry in 0.5% Triton X-100 in 10 mM Tris·HCl, pH 7.4, with 3 mM $MgCl_2$ and 10 mM NaCl (binding buffer). hMac-2 RNA (1–2 μg) transcribed *in vitro* was translated in reticulocyte lysate (Promega) in a 50-μl reaction mixture containing 40 μCi (1 μCi = 37 kBq) of [^{35}S]methionine. At the end of the translation 20 μl of the reaction mixture was diluted in 1 ml of binding buffer and incubated with 0.2 ml of the laminin-Sepharose slurry overnight at 4°C with gentle agitation, in either the presence or the absence of competing sugars. After the beads had been washed with binding buffer, the bound proteins were eluted by boiling in sample buffer and then analyzed by SDS/PAGE.

The human cell lines THP-1 (monocytic leukemia) and HL60 (promyelocytic leukemia) were obtained from the American Type Culture Collection (Rockville, MD). The promyelocytic cell line HL60 was induced to differentiate toward the macrophage phenotype by treatment with 10^{-7}M phorbol 12-myristate 13-acetate (PMA) for 48 hr (11) and toward the myelocyte phenotype by treatment with 1.25% (vol/vol) dimethyl sulfoxide for 5 days (12).

RESULTS

Molecular Cloning of hMac-2 cDNA. Screening of a human activated monocyte cDNA library with the mouse Mac-2 probe under reduced stringency resulted in the isolation of several clones. The restriction map of the clone with the longest insert, hMac2.2, is shown in Fig. 1. Preliminary sequencing of this insert indicated that though it was highly similar to Mac-2, it did not extend sufficiently 5′ to include the translational start site. To obtain a clone with further 5′ sequence, the 660-bp *Eco*RI fragment of hMac2.2 was used to screen a cDNA library from the human colon carcinoma cell line HT-29 (which expresses hMac-2; data not shown) under high-stringency conditions. Of the clones thus obtained one, hMac2.3, was found to include the translational start site. The inserts from these clones were subcloned in the vector pBSK and used for subsequent experiments.

Sequence of hMac-2 cDNA. The nucleotide sequence and predicted amino acid sequence of the hMac-2 cDNA is shown in Fig. 2. We have obtained a total of 935 bp of cDNA sequence. Given that the size of the corresponding mRNA is approximately 2 kilobases (kb) (see below), the sequence lacks about 1 kb of the 5′ untranslated region. The single long

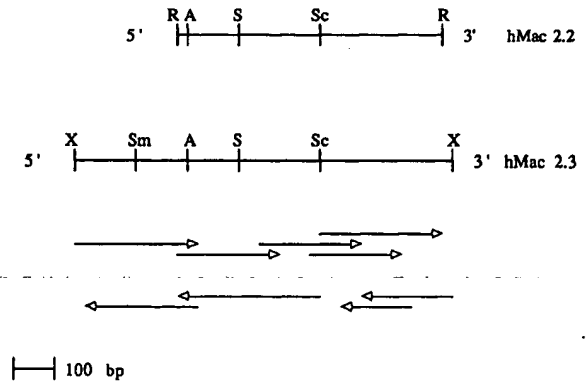

FIG. 1. Restriction map of hMac2 cDNA clones. Vertical lines indicate restriction sites: R, *Eco*RI; A, *Acc* I; S, *Sph* I; Sc, *Sca* I; Sm, *Sma* I; X, *Xho* I. Horizontal arrows indicate direction and extent of sequencing.

```
   1 GGAGCCAGCCAACGAGCGGAAAATGGCAGACAATTTTTCGCTCCATGATGCGTTATCTGG 60
   1                   M  A  D  N  F  S  L  H  D  A  L  S  G  13

  61 GTCTGGAAACCCAAACCCTCAAGGATGGCCTGGCGCATGGGGGAACCAGCCTGCTGGGCA 120
  14 S  G  N  P  N  P  Q  G  W  P  G  A  W  G  N  Q  P  A  G  Q  33

 121 GGGGCTACCCAGGGGCTTCCTATCCTGGGGCCTACCCGGGGCAGGCACCCCCAGGGCTTA 180
  34 G  L  P  R  G  F  L  S  W  G  L  P  R  A  G  T  P  R  A  Y  53

 181 TCCTGGACAGGCACCTCCAGGCGCCTACCCTGGAGCACCTGGAGCTTATCCCGGAGCACC 240
  54 P  G  Q  A  P  P  G  A  Y  P  G  A  P  G  A  Y  P  G  A  P  73

 241 TGCACCTGGAGTCTACCCAGGGCCACCCAGCGGCCCTGGCTACCCATCTTCTGGACAGCC 300
  74 A  P  G  V  Y  P  G  P  P  S  G  P  G  Y  P  S  S  G  Q  P  93

 301 AAGTGCCCCCGGAGCCTACCCTGCCACTGGCCCCTATGGCGGCCCCTGCTGGGCCACTGAT 360
  94 S  A  P  G  A  Y  P  A  T  G  P  Y  G  A  P  A  G  P  L  I  113

 361 TGTGCCTTATAACCTGCCTTTGCCTGGGGGAGTGGTGCCTCGCATGCTGATAACAATTCT 420
 114 V  P  Y  N  L  P  L  P  G  G  V  V  P  R  M  L  I  T  I  L  133

 421 GGGCACGGTGAAGCCCAATGCAAACAGAATTGCTTTAGATTTCGAAAGAGGGAATGATGT 480
 134 G  T  V  K  P  N  A  N  R  I  A  L  D  F  Q  R  G  N  D  V  153

 481 TGCCTTCGACTTTAACCCACGCTTCAATGAGAACAACAGGAGAGTCATTGTTTGCAATAC 540
 154 A  F  H  F  N  P  R  F  N  E  N  N  R  R  V  I  V  C  N  T  173

 541 AAAGCTGGATAATAACTGGGGAAGGGAAGAAAGACAGTCGGTTTTCGCATTTGAAAGTGG 600
 174 K  L  D  N  N  W  G  R  E  E  R  Q  S  V  F  P  F  E  S  G  193

 601 GAAACCATTCAAAATACAAGTACTGGTTGAACCTGACCACTTCAAGGTTGCAGTGAATGA 660
 194 K  P  F  K  I  Q  V  L  V  E  P  D  H  F  K  V  A  V  N  D  213

 661 TGCTCACTTGTTGCAGTACAATCATCGGGTTAAAAAACTCAATGAAATCAGCAAACTGGG 720
 214 A  H  L  L  Q  Y  N  H  R  V  K  K  L  N  E  I  S  K  L  G  233

 721 AATTTCTGGTGACATAGACCTCACCAGTGCTTCATATACCATGATATAATCTGAAAGGGG 780
 234 I  S  G  D  I  D  L  T  S  A  S  Y  T  M  I                 248

 781 CAGATTAAAAAAAAAAAAAAAAGAATCTAAACCTTACATGTGTAAAGGTTTCATGTTCACT 840

 841 GTGAGTGAAAATTTTTACATTCATCAATATCCCTCTTGTAAGTCATCTACTTAATAAATA 900

 901 TTACAGAGAAAAAAAAAAAAAAAAAAAAAAAAAAA 935
```

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the hMac-2 cDNA. The repeated hexapeptide as well as the motif found in S-type lectins is underlined.

open reading frame commences with the ATG at nucleotide 23, which is surrounded by a consensus translational initiation sequence (13) and terminates with the TAA codon at nucleotide 767. It encodes a 248-amino acid protein with a predicted molecular weight of 26,294 which has two domains: an amino-terminal portion (extending up to amino acid 137) which is proline- and glycine-rich and contains the motif Pro-Gly-Ala-Tyr-Pro-Gly (PGAYPG) repeated (as such or with minor modifications) five times and a carboxyl-terminal portion containing a consensus S-type lectin motif (14). It is worth noting that hMac-2, like its murine homolog, lacks an amino-terminal signal peptide or any other region of significant hydrophobicity. The 3′ untranslated region of the hMac-2 cDNA is distinctly A+T rich and there is a long poly(A) stretch between positions 787 and 801, well upstream of the poly(A) tail at the 3′ end. The former is unlikely to be a cloning artifact since it is present in clones isolated from both the monocyte and HT29 libraries.

A comparison of the predicted amino acid sequences of the mouse and human Mac-2 proteins is shown in Fig. 3. There is an overall amino acid sequence identity of 77%. If allowance is made for conservative amino acid changes, the similarity of the two sequences is 85%. The maximum conservation of primary structure is seen in the lectin domain. The repeated motif PGAYPG in the amino-terminal domain is seen in both proteins, but hMac-2 has fewer repeats, accounting for its smaller size.

Characterization of hMac-2 Synthesized *in Vitro*. The hMac-2 protein was synthesized *in vitro* by transcription off the T7 promoter on CDM8 and translation of the mRNA in a reticulocyte lysate. The protein so produced was immunoprecipitated specifically by the monoclonal antibody M3/38 directed against the murine Mac-2 antigen (1) and not by an isotype-identical control antibody (Fig. 4, lanes 3 and 4) and comigrated with the protein immunoprecipitated by this

```
  1 MADNFSLHDALSGSGNPNPQGWPGAWGNQPAGQGLPRGFLSWGLPRAGTP 50
    |||.|||:|||.||||||||:||||||||:::.|.| : .. :.| .:.|
  1 MADSFSLNDALAGSGNPNPQGYPGAWGNQPGAGGYPGAAYPGAYPGQAPP 50

 51 RAYPGQAPPGAYPG..APGAYPGAPAPGVYPGPPSGPGYPSSGQ..... 92
    |||||||||||||  :|:||||:.|||.||||....:||:|..
 51 GAYPGQAPPGAYPGQAPPSAYPGPTAPGAYPGPTAPGAYPGSTAPGAFPG 100

 93 .........PSAPGAYPATGPYGAPAGPLIVPYNLPLPGGVVPRMLITILG 134
         |||||:|||.||||.||||||.|||:|||||||:|||||||:|
101 QPGAPGAYPSAPGGYPAAGPYGVPAGPLTVPYDLPLPGGVMPRMLITIMG 150

135 TVKPNANRIALDFQRGNDVAFHFNPRFNENNRRVIVCNTKLDNNWGREER 184
    |||||||||.|||.|||||||||||||||||||||||||| |||||:|||
151 TVKPNANRIVLDFRRGNDVAFHFNPRFNENNRRVIVCNTKQDNNWGKEER 200

185 QSVFPFESGKPFKIQVLVEPDHFKVAVNDAHLLQYNHRVKKLNEISKLGI 234
    ||.|||||||||||||:|||||||||||||||||||:|.|.|||.|||
201 QSAFPFESGKPFKIQVLVEADHFKVAVNDAHLLQYNHRMKNLREISQLGI 250

235 SGDIDLTSASYTMI 248
    ||||.||||...||
251 SGDITLTSANHAMI 264
```

FIG. 3. Comparison of the predicted amino acid sequence of hMac-2 (upper line) with that of Mac-2 (lower line). Between sequences, vertical lines indicate amino acid identity and dots indicate similarity (:, high identity score; ., moderate identity score).

antibody from a metabolically labeled lysate of the human monocyte cell line THP-1 (Fig. 4, lanes 1 and 2). The *in-vitro*-synthesized protein was also precipitated by the desialylated glycoprotein asialofetuin coupled to Sepharose (Fig. 4, lane 7) but not by Sepharose coupled to the nonglycosylated protein myoglobin (not shown). This binding was unaffected by 100 mM glucose but was inhibited by an equivalent concentration of galactose (Fig. 4, lanes 5 and 6).

hMac-2 Synthesized *in Vitro* Binds Laminin. As shown in Fig. 5, lane 1, purified laminin coupled to Sepharose bound to and precipitated hMac-2 protein synthesized *in vitro*. This binding was not inhibited by the presence of 100 mM glucose but was significantly reduced by galactose at the same concentration (Fig. 5, lanes 2 and 3). Similar results were obtained when murine Mac-2 protein synthesized *in vitro* was used (unpublished data).

Expression of hMac-2 in Various Cell Lines. The hMac-2 cDNA was used to probe a Northern blot of total cellular RNAs from various human cell lines. The results are shown in Fig. 6 and indicate that the hMac-2 probe recognizes an approximately 2-kb mRNA which is expressed at a low level in the monocytic cell line THP-1 and at a higher level in the promyelocytic cell line HL60. Expression increases in HL60 when it is induced to differentiate, especially by PMA. hMac-2 expression can also be detected in the fibroblast cell line SL68 and in various epithelial cell lines, including HeLa, SCC, HT-29, and CaCo 2, while it is not detectable in the lymphoid cell line BJAB (data not shown).

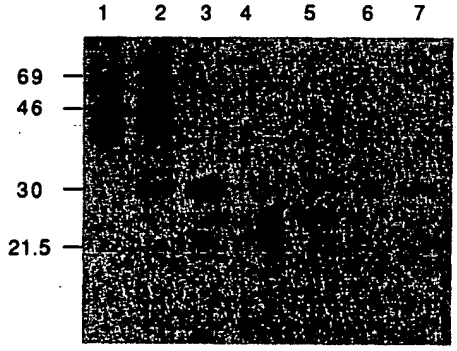

FIG. 4. Characterization of *in-vitro*-synthesized hMac-2 protein by immunoprecipitation of metabolically labeled cell lysates and SDS/PAGE. Lanes 1 and 2 show immunoprecipitates of a lysate of THP-1. In lane 2 M3/38, the monoclonal antibody to Mac-2, was used; in lane 1 an isotype-identical control antibody was used. Lanes 3 and 4 show immunoprecipitates of *in-vitro*-synthesized hMac-2 protein, with M3/38 in lane 3 and control antibody in lane 4. Lanes 5–7 show precipitation of *in-vitro*-synthesized hMac-2 protein by asialofetuin coupled to Sepharose. The precipitations were carried out in the absence of competing sugar (lane 7), in the presence of 100 mM galactose (lane 6), or in the presence of 100 mM glucose (lane 5). The numbers to the left indicate molecular weights $\times 10^{-3}$.

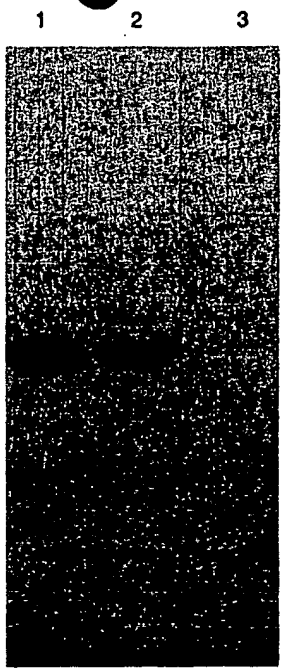

FIG. 5. Binding of [^{35}S]methionine-labeled *in-vitro*-translated hMac-2 protein to laminin-Sepharose: SDS-PAGE analysis. Lane 1, binding carried out in the absence of competing sugar; lane 2, binding in the presence of 100 mM glucose; lane 3, binding in the presence of 100 mM galactose.

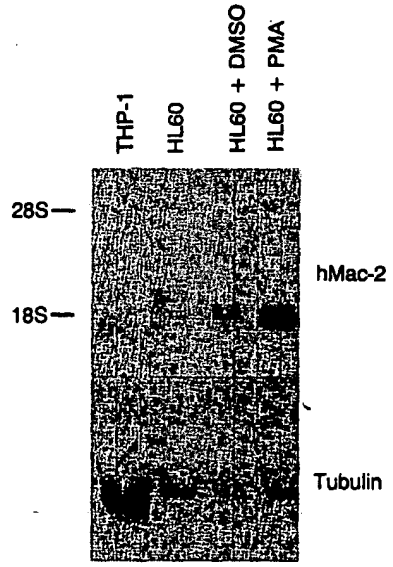

FIG. 6. Northern blot of total cellular RNA from two cell lines hybridized to hMac2 and tubulin probes. HL60 cells were grown in the presence of dimethyl sulfoxide (DMSO) or PMA as indicated. In the lane marked THP-1 30 μg of RNA was loaded; all other lanes were loaded with 5 μg of RNA. On the left, 28S and 18S indicate the positions of the corresponding ribosomal RNAs.

DISCUSSION

The properties of the murine Mac-2 antigen which we previously described indicated clearly that it is a galactose-specific lectin (2). Its marked up-regulation in inflammatory macrophages in comparison to resident tissue macrophages suggested that it might be involved in the inflammatory process. Its close homology to a rat IgE-binding protein (6) raised the possibility that it may have a role in the regulation of IgE biosynthesis or function. Several other proteins with sizes and galactose-binding properties similar to Mac-2 have been described. cDNAs encoding the lectins described by Jia and Wang (3) and Raz *et al.* (4) have been cloned and their sequences are identical to the sequence of Mac-2 cDNA. Various functions have been attributed to the individual members of this group of proteins, some of which have clear relevance to human disease. To obtain further clues to the function of Mac-2 and its relatives and because of their potential clinical importance, we have cloned the human homolog of these various rodent proteins and we have designated it hMac-2.

The primary structures of Mac-2 and hMac-2 are highly conserved, with 85% of the amino acids being similar. Like the murine protein, hMac-2 demonstrates a clear two-domain organization and the conservation of primary structure is especially marked in the carboxyl-terminal lectin domain in which there are long, uninterrupted stretches of amino-acid sequence identity. The amino-terminal half of the protein contains the motif PGAYPG seen in Mac-2 but the number of repeats of this sequence is smaller in the human protein. While the exact function of this repetitive sequence is not clear, its evolutionary conservation suggests that it has some important role.

As was observed in the case of Mac-2, the predicted amino acid sequence of hMac-2 does not contain a signal peptide or transmembrane domain, raising questions regarding the mechanism by which these proteins are localized to the cell surface. We have previously demonstrated the existence of two distinct Mac-2 cDNAs which are alternatively spliced at their 5′ ends upstream of the initiation codon, and we suggested that one of the alternatively spliced forms encoded a signal peptide (2). The limited sequence that we have obtained for the 5′ untranslated region of the hMac-2 cDNA does not bear similarity to the corresponding region of either of the murine cDNAs, suggesting that this region is not of sufficient functional significance to be evolutionarily conserved. In keeping with this observation we have now found that neither of the alternatively spliced Mac-2 cDNAs encodes a protein with a signal peptide. Secretion appears to occur by a signal-peptide-independent mechanism, and some of the secreted protein is retained on the cell surface by binding to galactose-containing glycoproteins or glycolipids (B.J.C. and S.P., unpublished results). We assume that hMac-2 utilizes a similar mechanism for export and surface localization. Mac-2 and hMac-2, being secreted proteins with the ability to bind to carbohydrates on the cell surface and on extracellular molecules, are well suited to serving a "bridging" function in cell–cell and cell–substratum interactions.

Given the extent of conservation of the primary structure of Mac-2 and hMac-2, it is not surprising that the two proteins have similar antigenic and ligand-binding properties. hMac-2 is specifically recognized in immunoprecipitations and Western analyses by the monoclonal antibody M3/38, which was raised against the murine protein. This finding indicates that M3/38 could be used for the detection of hMac-2 in human cells and tissues and paves the way for the use of this reagent in investigations of the involvement of hMac-2 in human disease states. Like the murine protein, hMac-2 has the properties of a galactose-specific lectin, as demonstrated by its galactose-inhibitable binding t the desialylated glycoprotein asialofetuin.

In keeping with its homology to the rat IgE binding protein, we have demonstrated that Mac-2 is able to bind specifically to murine IgE and that this binding is inhibited by galactose (2). These findings were also obtained independently by Laing *et al.* (15) for carbohydrate-binding protein 35. The data from these studies did not indicate whether this binding occurred through a galactose-containing sugar side chain on IgE or whether a distinct IgE-binding site on Mac-2 was involved. Preliminary experiments from our laboratory indicate that hMac-2 binds to both human and murine IgE at a low but detectable level. The mechanism and significance of this binding are not yet known.

Recently, Woo *et al.* (7) have identified Mac-2 as being the major nonintegrin laminin-binding protein expressed by murine thioglycollate-elicited peritoneal macrophages. This finding has important implications for the role of Mac-2 and hMac-2 in the inflammatory process. One of the early responses in inflammation is the induction of cell adhesion molecules such as ICAM-1 (16), INCAM-110 (17), VCAM-1 (18), and ELAM-1 (19) on the surface of vascular endothelial cells at the site of tissue injury. These molecules allow circulating monocytes, neutrophils, and lymphocytes to adhere to and subsequently make their way between the endothelial cells, through the basement membrane, and into the tissue space. During this process the leukocytes must be capable of transiently interacting with the basement membrane. One of the major components of the basement membrane is laminin, a large multisubunit protein which has sites for binding to cells and to other constituents of the basement membrane (20). While the $\alpha_6\beta_1$ integrin, a member of the integrin family of transmembrane receptors (21), is likely t play a central role in the adhesion of macrophages to basement membrane laminin (22), a secreted protein such as Mac-2, which has the ability to bind to laminin and to the cell surface, may be of importance in directing cell movement towards the basement membrane. Mac-2 may also facilitate the actual adhesion process. The results of Woo *et al.* (7) and the experiments reported here indicated that both Mac-2 and hMac-2 bind to laminin through the numerous terminal galactose residues which have been demonstrated on the sugar side chains of the latter protein (23). Such a protein–carbohydrate interaction could stabilize the protein–protein interaction involved in the integrin-mediated binding of cells to laminin.

In addition to potentially having a role in inflammation, Mac-2 may be involved in noninflammatory processes. Raz and colleagues showed that the level of expression of L-34 (Mac-2) on melanoma and fibrosarcoma cells correlated with their metastatic ability (24) and that a monoclonal antibody t L-34 inhibited the formation of metastases by these cells (25). Since the process of metastasis is analogous to inflammatory exudation in that it involves the crossing of basement membranes by circulating tumor cells, the laminin-binding property of Mac-2 provides a plausible explanation for these results.

The pattern of tissue-specific expression of hMac-2 is very similar to that of Mac-2; namely, it is expressed in cells of the monocyte-macrophage series, in various epithelial cells, and in fibroblasts, while expression is not detectable in lymphoid cells. It is interesting that induction of macrophage differentiation in the promyelocytic line HL60 results in a significant increase in hMac-2 expression. A similar increase in Mac-2 expression has been correlated with the state of differentiation of various murine macrophage cell lines (26). Mac-2 expression reaches its highest level in thioglycollate-elicited peritoneal inflammatory macrophages (1, 2). We have not yet examined the expression of hMac-2 in human macrophages activated by inflammatory stimuli, but the PMA-induced

upregulation of hMac-2 in HL60 suggests that a similar increase in expression may occur during the inflammatory activation of macrophages, since stimulation of protein kinase C is known to be involved in this process (27). It is an intriguing possibility that interaction between the adhesion molecules induced on vascular endothelium and cognate receptors on the surface of circulating monocytes may be responsible for the massive upregulation of Mac-2 in inflammatory macrophages. With the availability of the cloned Mac-2 and hMac-2 cDNAs and the antibody to the proteins, we are in a position to examine such possibilities more closely. Involvement of hMac-2 in various disease states will also now be amenable to investigation.

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